

**INTERACTIONS BETWEEN THE EXOCRINE AND ENDOCRINE
PANCREAS**

PhD Thesis

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CONTENTS

ABBREVIATIONS.....	3
List of full papers cited in the Thesis	5
List of full papers not related to the subject of Thesis	6
1. INTRODUCTION.....	7
1.1 Pancreatic exocrine insufficiency in type 2 diabetes mellitus.....	7
1.2 Fat accumulation in the liver, pancreas and pericardium in diabetes	8
2. PATIENTS AND METHODS	10
I. Prevalence of exocrine pancreatic insufficiency in type 2 diabetes mellitus with poor glycemic control	10
Patients	10
Methods	10
Statistical analysis	11
II. Evaluation the incidence rate of NAFLD, NAFPD and PAT size, and the effect of metformin therapy on NAFLD, NAFPD and PAT in NODM	11
Patients	11
Methods	11
Statistical analysis	12
III. Current concepts of PEI in diabetes mellitus. Systematic review	12
Search strategy	12
Study Selection	13
3. RESULTS	14
I. Prevalence of exocrine pancreatic insufficiency in type 2 diabetes mellitus with poor glycemic control	14
II. Evaluation the incidence rate of NAFLD, NAFPD and PAT size, and the effect of metformin therapy on NAFLD, NAFPD and PAT in NODM	16
III. Current concepts of PEI in diabetes mellitus. Systematic review	22

Prevalence of exocrine pancreatic insufficiency in diabetes mellitus	22
Prevalence of morphologic changes of the exocrine pancreas in diabetes mellitus	25
4. DISCUSSION	27
I. Prevalence of exocrine pancreatic insufficiency in type 2 diabetes mellitus with poor glycemic control.....	27
II. Evaluation the incidence rate of NAFLD, NAFPD and PAT size, and the effect of metformin therapy on NAFLD, NAFPD and PAT in NODM	30
III. Current concepts of PEI in diabetes mellitus. Systematic review	33
Pathophysiology of PEI in diabetes mellitus	33
Symptoms of PEI in diabetes	34
Diagnosis of PEI	35
Therapy	36
5. NEW RESULTS ESTABLISHED IN THE THESIS	38
I. Prevalence of exocrine pancreatic insufficiency in type 2 diabetes mellitus with poor glycemic control.....	38
II. Evaluation the incidence rate of NAFLD, NAFPD and PAT size, and the effect of metformin therapy on NAFLD, NAFPD and PAT in NODM	38
III. Current concepts of PEI in diabetes mellitus. Systematic review	38
6. SUMMARY	39
7. ACKNOWLEDGMENTS	42
8. REFERENCES	43
9. ANNEXES	60
Annex I.	60
Annex II.	66
Annex III.	74

ABBREVIATIONS

ADA – American Diabetes Association

BMI – body mass index

CCK-PZ – cholecystokinin-pancreozymin

CFA – coefficient of fat absorption

CP – chronic pancreatitis

CT – computer tomography

DM – diabetes mellitus

ERCP – endoscopic retrograde cholangiopancreatography

FE-1 – fecal elastase-1

FIP – International Pharmaceutical Federation

GI – gastrointestinal

GIP – glucose-dependent insulintropic polypeptide

GLP-1 – glucagon-like peptide-1

HbA1c – glycosylated haemoglobin

HOMA-IR – homeostatic model assessment-estimated insulin resistance

HU – Hounsfield Unit

IDDM – insulin-dependent diabetes mellitus

MRI – magnetic resonance imaging

MRCP – magnetic resonance cholangiopancreatography

NAFLD – non-alcoholic fatty liver disease

NAFPD – nonalcoholic fatty pancreas disease

NASH – non-alcoholic steatohepatitis

NODM – new-onset type 2 diabetes mellitus

OR – odds ratio

PAT – pericardial adipose tissue

PE-1 – pancreatic elastase-1

PEI – pancreatic exocrine insufficiency

PERT – pancreatic enzyme replacement therapy

pre-DM – prediabetes mellitus

PSCs – pancreatic stellate cells

ROI – region of interest

SCT – secretin-cerulein test

SD – standard deviation

T2DM – type 2 diabetes mellitus

US – ultrasonography

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1. INTRODUCTION

1.1. Pancreatic exocrine insufficiency in type 2 diabetes mellitus

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 [1]. Pancreatic exocrine insufficiency (PEI) is defined by a deficiency of exocrine pancreatic enzymes resulting in an inability to maintain normal digestion [2]. The primary function of pancreatic enzymes is the hydrolysis of proteins (trypsinogens, proelastase, mesotrypsin), carbohydrates (alfa-amylase), lipids (lipase) and nucleotids (DNase, RNase). 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 [3].

PEI has been demonstrated to be present in a considerable percentage (10–74%) of patients with diabetes mellitus (DM) [4, 5]. 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 [6,7]. 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 [8]. However, the significance of this findings was questioned and it is not clear, whether the presence of diabetes causes any symptoms or requires any treatment [9]. Abdominal symptoms such as nausea, bloating, diarrhea, steatorrhea, and weight loss can often occur in diabetic patients [4]. These symptoms may be attributed to the side-effects of the metformin they are taking, the autonomic neuropathy on bowel function, small bowel bacterial overgrowth, celiac disease, or PEI. Impairments of the exocrine pancreatic function seem to be a frequent complication of DM; however, they are largely overlooked. Greater knowledge and awareness are required in testing and diagnosing this condition.

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 [10]. However, the significance of an exocrine dysfunction in DM has recently been questioned [9], 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 [11].

PEI has not been accurately determined to date in relation to the level of glycemic control. The level of pancreatic elastase-1 (PE-1) has been reported to be lower [12] or not to differ [13] 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% [14].

1.2. Fat accumulation in the liver, pancreas and pericardium in diabetes

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]) [15]. 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 [16–20]. There is a high prevalence of NAFLD in patients with T2DM (64–69%) [16, 21–24] and dyslipidemia (20–81%) [25, 26]. Obesity, T2DM, and dyslipidemia are risk factors for the development of NAFLD [22, 23, 27, 28]. 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 [29]. Moreover, it seems that the male gender is presumably a further risk factor for NAFLD [16]. If left untreated, NAFLD may progress through steatohepatitis to cirrhosis and hepatocellular carcinoma [30, 31].

Besides the exocrine function, the pancreas morphology has been revealed by ultrasonography (US), computer tomography (CT), endoscopic retrograde pancreatography or autopsy to be altered in many patients with DM [32–35]. 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 [36]. Previous studies have suggested a 16%–35% prevalence of fatty pancreas in the general population [37, 38]. It seems that age [39], obesity, hyperglycemia, and dyslipidemia are risk factors for NAFPD [38, 40–42]. NAFPD may increase the risk for the development of metabolic syndrome by causing inflammation [43], impaired pancreatic beta cell function, and finally hyperglycemia [44]. This relationship may explain the presence of T2DM [45] in 6.9–12.6% of patients with NAFPD [37, 38]. Several studies have suggested that insulin resistance is associated with pancreatic fat accumulation [40–42, 46, 47], nonalcoholic steatohepatitis

(NASH) [40, 48], and pre-DM [41]. Higher pancreatic triglyceride content in obesity can be detected by proton magnetic resonance spectroscopy [49], CT [50, 51] or magnetic resonance imaging (MRI) [52] even before the development of T2DM [49]. It has been demonstrated that obesity may lead to pancreatic ductal adenocarcinoma through NAFLD [43, 53, 54]. Evidence suggests that NAFLD plays a role in T2DM, pancreatic exocrine dysfunction, acute pancreatitis [55, 56], pancreatic cancer, and the formation of pancreatic fistulas after pancreatic surgery [57]. NAFLD and NAFLD are associated with each other because pancreatic fat formation is related to NASH and is a significant predictor of the presence of NAFLD [58]. The elevation of liver transaminases may suggest the presence of NAFLD or NASH. 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 [59]. In contrast to the liver, no biochemical marker is available for diagnosing NAFLD. 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 NAFLD are hampered by obesity and bloating. Further, in prediabetic and T2DM patients, the amount of PAT is significantly higher compared with that in normoglycemic patients [60, 61]. Previous reviews have demonstrated that, besides epicardial adipose tissue, PAT is another risk factor for the development of cardiovascular disease in T2DM patients [62].

Our aims were

- I. to assess the possible relationship between T2DM with poor glycemic control ($HbA1c \geq 7.0\%$), an exocrine pancreatic insufficiency and alterations in pancreatic morphology;
- II. to evaluate the incidence rate of NAFLD, NAFLD and PAT size, and the effect of metformin therapy on NAFLD, NAFLD 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.

2. PATIENTS AND METHODS

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

Patients

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] $\geq 7\%$), 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 [63]. Cases with type 3c DM, i.e. diabetes secondary to exocrine pancreatic diseases, were excluded. The patients were not operated on the pancreas.

All patients provided their written informed consent to participation. The study protocol was in full accordance with the most recent revisions of the Helsinki Declaration and was approved by the ethics committee at the University of Szeged.

Methods

Fecal PE-1 was determined through the use of monoclonal antibodies (ScheBo Biotech AG, Giessen, Germany). PE-1 concentrations $> 200 \mu\text{g/g}$ in the stools indicated a normal exocrine pancreatic function, concentrations between 100 and 200 $\mu\text{g/g}$ indicated a mild exocrine pancreatic insufficiency, and concentrations $< 100 \mu\text{g/g}$ denoted a severe exocrine pancreatic insufficiency [64].

Abdominal US and CT were performed to detect the characteristic morphological features of CP. US was applied for the detection of pancreatic steatosis. The liver, or the kidney if the liver was hyperechogenic, was used as reference point; echogenicity of the pancreas higher than that of the liver or kidney parenchyma was an indicator of pancreatic

steatosis [65]. The thickness of the pancreas was measured by US just across the spine [32, 66]. All US examinations were carried out by the same gastroenterologist, who was blind to the other results on the patients.

Statistical analysis

The experimental data were analyzed statistically with the independent-samples t-test, the Mann-Whitney U test, the chi-square test. The linear association between two variables was compared by Pearson-correlation analysis. P values <0.05 were accepted as being statistically significant. Statistical data are expressed as means \pm standard deviation (SPSS 13.0 for Windows).

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

Patients

The diagnosis of T2DM was made in accordance with the ADA criteria [67]. 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.

Methods

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 [68]. 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 [69, 70].

All the participants provided written informed consent. The study protocol was in full accordance with the most recent revisions of the Helsinki Declaration and was approved by the ethics committee at the University of Szeged.

Statistical analysis

Continuous measures are summarized and presented as means and standard deviations. Categorical data are presented as percentages. The two-sample t-test and paired samples t-test were used to determine differences between continuous parameters. Non-normally distributed data were log transformed. Data were processed with SPSS 22.0 (Armonk, NY), and a level of $P < 0.05$ was considered statistically significant.

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

Search strategy

The systematic review was conducted following the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement [71]. A systematic search was performed in 3 databases, Pubmed, Embase and Cohrane 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.

Study selection

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 III/C diabetes 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 judgement, then a committee of three other researchers was invited to draw a conclusion.

3. RESULTS

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

A total of 106 type 2 diabetic patients were recruited between April 1, 2012 and June 30, 2013. Five patients were excluded, because morphological examinations revealed the characteristic features (calcifications and dilation of the main pancreatic duct) of advanced CP, all of them with poor glycemic control and with a decreased PE-1 concentration. Consequently, 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 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 (Table 2).

TABLE 1. MORPHOLOGICAL FINDING OF THE PANCREAS

Parameters	Group A	Group B	P
Number of patients	59	42	-
Male/Female	25/34	22/20	-
Mean age (years)	63.6 ± 10.4	57.1 ± 11.2	$p<0.008$
Duration of DM (mean \pm SD) (years)	13.2 ± 8.9	8.3 ± 7.9	$p<0.006$
BMI (mean \pm SD)	29.74 ± 5.65	28.43 ± 5.23	$p=0.278$
Mean BMI of patients with decreased PE-1 (mean \pm SD)	29.99 ± 4.58	32.29 ± 5.29	$p=0.658$

Treatment of diabetes (oral antidiabetics/insulin/diet only)	12/47/0	23/15/4	-
Fasting plasma glucose (mean±SD) (mmol/l)	10.56±3.64	7.03±1.65	<i>p</i> <0.001
Mean HbA1c (range) (%)	8.3 (7-11.6)	6.2 (5-6.9)	<i>p</i> <0.001

DM: diabetes mellitus; BMI: body mass index; HbA1c: glycosylated haemoglobin; PE-1: pancreatic elastase-1.

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 decreased in overall 16.8% of the cases. There were no significant differences in BMI between the patients with a decreased or a normal PE-1 concentration within Group A or B (Table 2).

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) (Fig. 1).

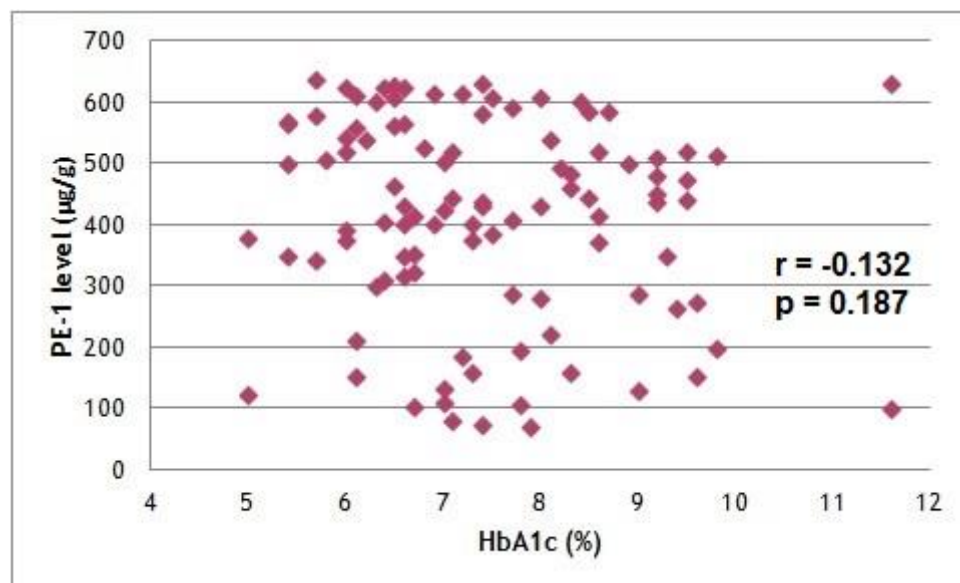


Figure 1. The PE-1 level was not correlated with HbA1c.

HbA1c: glycosylated haemoglobin; PE-1: pancreatic elastase-1.

US revealed pancreatic steatosis in 35 patients: 19 in Group A and 16 in Group B. Pancreatic steatosis was less frequent in Group A (32.2%) than in Group B (38.1%) (OR=0.77), but it was more common in patients with an abnormal PE-1 concentration as compared with those with a normal PE-1 concentration (OR=1.4) (Table 3). The PE-1 level was not correlated with the presence of pancreatic steatosis ($r=0.117$, $p=0.244$). There were no other morphological alterations of the pancreas in our patients.

TABLE 2. MORPHOLOGICAL FINDINGS OF THE PANCREAS

Morphology of pancreas	Group A (59 cases)	Group B (42 cases)	P values
normal	39 (66.1%)	20 (47.6%)	$p=0.063$
steatosis	19 (32.2%)	16 (38.1%)	$p=0.54$
malignancy	1 (1.7%)	1 (2.4%)	$p=0.809$
thickness (mm)	14.8 ± 5.8	12.6 ± 3.7	$p=0.112$
no data (noncompliance)	0 (0%)	5 (11.9%)	-

The thickness of the pancreas did not differ significantly between Groups A and B, and there was no correlation between the PE-1 concentration and the size of the pancreas ($r=-0.061$, $p=0.670$).

The pancreatic morphology could not be evaluated in five subjects due to no compliance.

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; body mass index [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 (Table 3).

TABLE 3. AGE, BMI CHOLESTEROL AND TRIGLYCERIDE LEVELS IN PATIENTS WITH NEW-ONSET TYPE 2 DIABETES MELLITUS AND HEALTHY CONTROL SUBJECTS AT BASELINE

Parameters	Control (n=10)	NODM (n=17)	P values
Age (years)	53.9±13.8	55.3±10.8	p=0.521
BMI (kg/m ²)	30.0±4.7	31.8±5.1	p=0.437
Serum cholesterol (mmol/l)	5.1±0.9	5.7±1.9	p=0.187
Serum triglyceride (mg/dl)	3.3±2.7	3.1±1.8	p=0.830

BMI: body mass index.

Fasting blood glucose, HbA1c, serum insulin and cholesterol, and HOMA-IR decreased significantly after metformin therapy compared with the baseline values (Table 4). However, serum triglyceride and BMI did not change.

TABLE 4. THE CHANGES OF LABORATORY TEST RESULTS AND BMI FROM BASELINE VALUES TO 4 MONTHS AFTERWARDS DURING METFORMIN THERAPY IN PATIENTS WITH NEW-ONSET TYPE 2 DIABETES MELLITUS

Parameters	Baseline values (male: 8; female: 9)	4 months afterwards (male: 8; female: 9)	P values
Fasting blood glucose (mmol/l)	12.9±4.8	7.0±1.4	<i>p=0.001</i>
HbA1c (%)	9.6±2.8	6.7±0.8	<i>p<0.001</i>
Serum insulin (mU/l)	33.1±18.3	22.2±11.5	<i>p=0.003</i>
HOMA-IR (molar units)	16.6±9.0	7.1±3.1	<i>p<0.001</i>
Serum cholesterol (mmol/l)	4.8±1.0	4.4±1.0	<i>p=0.016</i>
Serum triglyceride (mg/dl)	2.9±1.1	2.4±1.3	p=0.299
BMI (kg/m ²)	31.8±5.1	31.6±4.4	p=0.757

HbA1c: glycosylated haemoglobin; HOMA-IR: homeostatic model assessment-estimated insulin resistance; BMI: body mass index.

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. NODM patients with and without NAFLD were compared (Table 5). 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 (Table 5).

TABLE 5. THE AMOUNT OF FAT IN THE LIVER, CHOLESTEROL, TRIGLYCERIDE, BMI, FASTING BLOOD GLUCOSE, HbA1c AND LIVER ENZYME VALUES IN NEW-ONSET TYPE 2 DIABETES MELLITUS PATIENTS WITH AND WITHOUT NAFLD AT BASELINE

Parameters	Patients with NAFLD (male: 6; female: 5)	Patients without NAFLD (male: 2; female: 4)	P values
The amount of fat in the liver (HU)	25.2 ± 12.7	55.6 ± 9.3	$p=0.002$
Serum cholesterol (mmol/l)	5.5 ± 1.1	5.8 ± 0.3	$p=0.002$
Serum triglyceride (mg/dl)	2.7 ± 1.4	2.9 ± 1.5	$p=0.776$
BMI (kg/m ²)	31.6 ± 5.2	29.2 ± 5.6	$p=0.233$
Aspartate aminotransferase (U/l)	20.6 ± 5.5	16.5 ± 3.0	$p=0.123$
Alanine aminotransferase (U/l)	24.9 ± 8.6	19.5 ± 6.5	$p=0.151$
Alkaline phosphatase (U/l)	76.8 ± 18.0	83.5 ± 24.4	$p=0.717$
Gamma glutamyltransferase (U/l)	43.9 ± 15.2	46.3 ± 25.6	$p=0.901$
Total bilirubin (μ mol/l)	8.1 ± 1.7	5.9 ± 2.5	$p=0.245$
Fasting blood glucose (mmol/l)	13.9 ± 5.9	12.3 ± 9.2	$p=0.882$
HbA1c (%)	9.4 ± 2.1	9.2 ± 2.8	$p=0.924$
HOMA-IR	17.8 ± 8.3	11.4 ± 9.5	$p=0.368$

HU: Hounsfield unit; NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; HbA1c: glycosylated haemoglobin; HOMA-IR: homeostatic model assessment-estimated insulin resistance.

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 ± 8.3 HU [$p=0.001$]) and significantly increased after metformin therapy compared with the baseline values (32.3 ± 17.7 vs. 47.3 ± 12.1 HU [$p=0.026$]) (Fig. 2). Only six patients (35.3%) had NAFLD after the 4-month metformin therapy according to the diagnostic criteria.

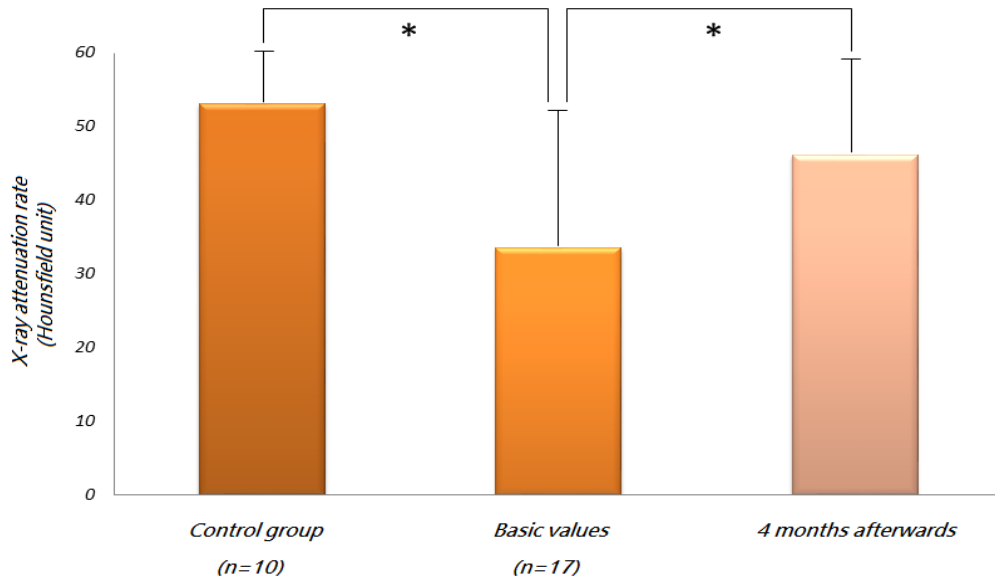


Figure 2. The radiation absorption of the liver in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. The X-ray attenuation rate during a native CT examination was measured in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. Data expressed as mean values \pm SD. * $P < 0.05$.

CT: computer tomography; SD: standard deviation.

The density of the liver was diffusely decreased in new-onset type 2 diabetic patients as compared with control. Metformin therapy increased liver density in the diabetic patients like the density in the control liver (Fig. 3).

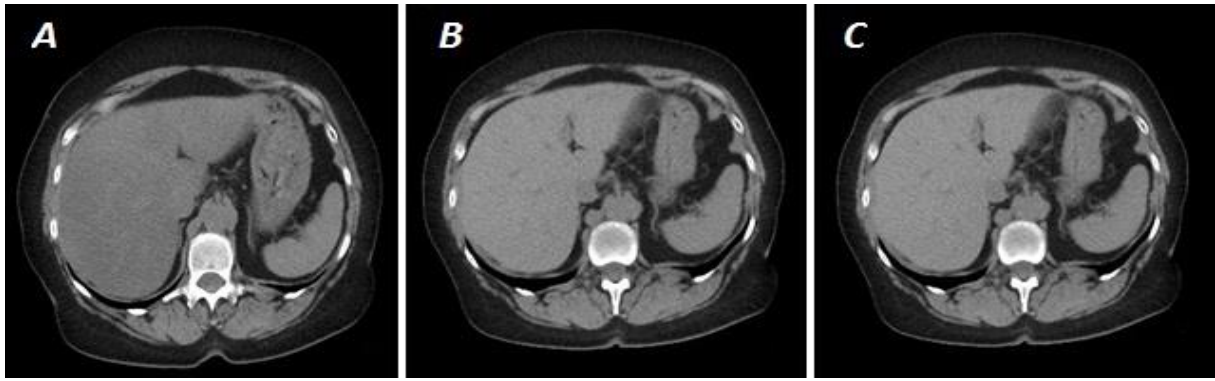


Figure 3. Typical plain CT images in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. The density of the liver was diffusely decreased in new-onset type 2 diabetic patients (A) as compared with control (C). Metformin therapy increased liver density in the diabetic patients (B) like the density in the control liver (C).

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 [$p = 0.178$]) (Fig. 4).

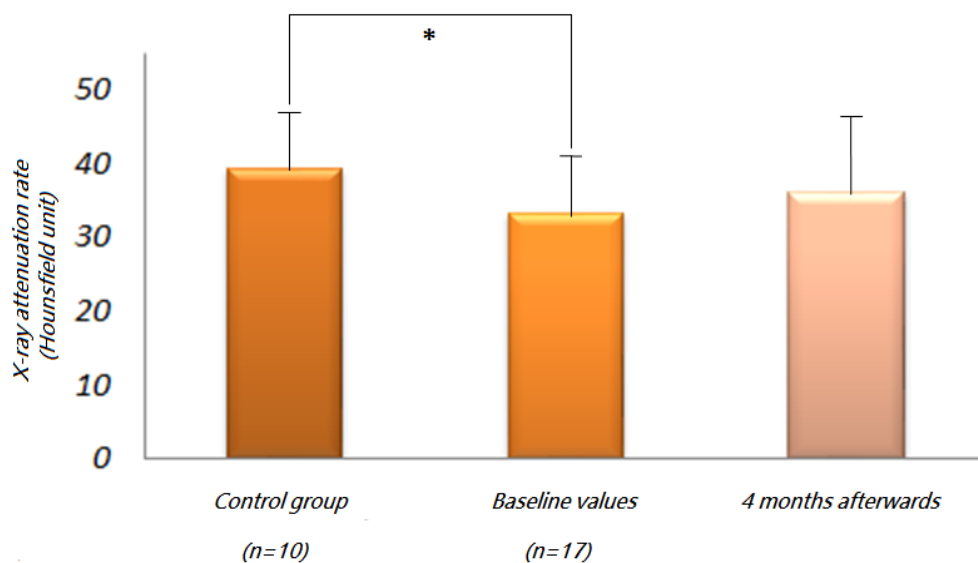


Figure 4. The radiation absorption of the pancreas in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. The X-ray attenuation rate during a native CT examination was measured in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. Data expressed as mean values \pm SD. * $P < 0.05$.

CT: computer tomography; SD: standard deviation.

PAT size was significantly larger in the patients with NODM compared with the control group (2143.1 ± 1036 vs. 1223.9 ± 312.9 mm² [$p = 0.008$]) and did not change significantly after the metformin treatment (2143.1 ± 1036 vs. 2048.2 ± 997 mm² [$p = 0.798$]) (Fig. 5).

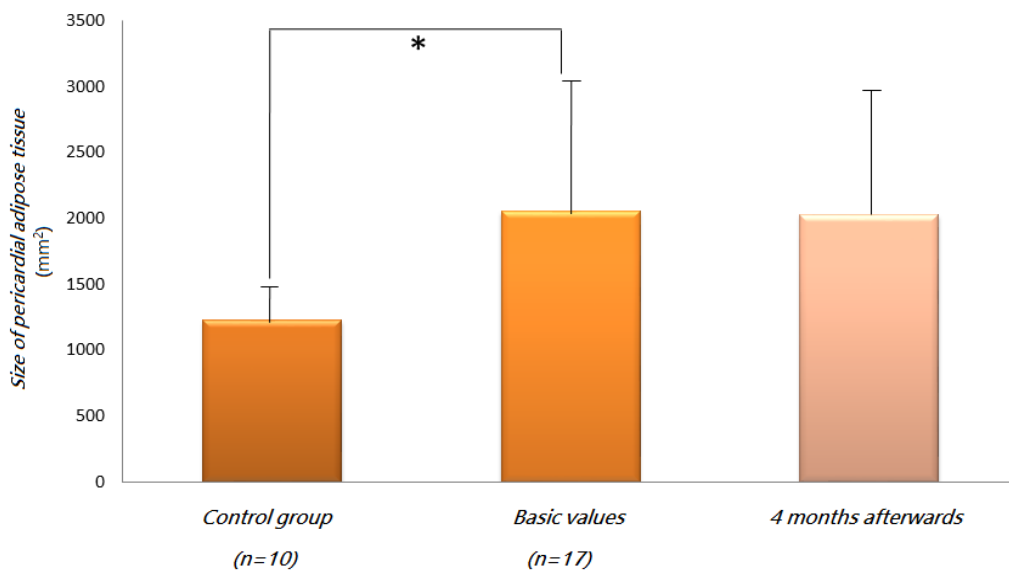


Figure 5. The size of pericardial adipose tissue in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. The X-ray attenuation rate during a native CT examination was measured in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. Data expressed as mean values \pm SD. * $P < 0.05$.

CT: computer tomography; SD: standard deviation.

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

Database searches yielded altogether 1055 articles (EMBASE: 67; PubMed: 701; Cochrane: 287). The flow-chart diagram (Fig. 6) shows the strategy and results of the study selection.

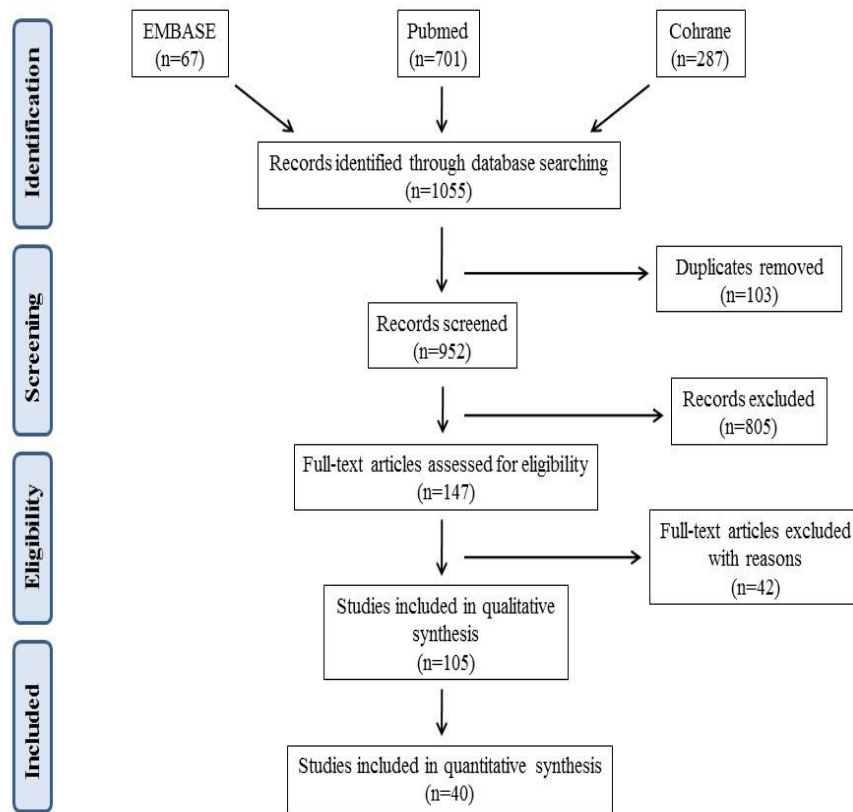


Figure 6. The flow-chart diagram shows the strategy of the study selection.

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 (Table 6a) [9, 72–79]. However, these studies were only limited to a small number of patients because direct pancreatic function tests are invasive, time-consuming and expensive.

TABLE 6A. RESULTS OF DIRECT PANCREATIC FUNCTION TESTS IN PATIENTS WITH DIABETES MELLITUS

Author	Subjects/diabetes type	Methods	Results
Pollard et al. 1943 [72]	13	Amylase and lipase after pancreozymin-secretin stimulation	62% reduced
Chey et al. 1963 [73]	50 diabetic patients; 13 juvenile type	Amylase and lipase after pancreozymin-secretin stimulation	Low amylase output in diabetes: 36%; in juvenile diabetes: 77%
Vacca et al. 1964 [74]	55 diabetic patients (22 insulin-treated)	Diastase and bicarbonate after secretin stimulation; fecal fat	73% abnormal; correlation with age, no correlation with fecal fat
Frier et al. 1976 [75]	20 type 1, 7 type 2, 13 controls	Stimulation with iv secretin and CCK-PZ	PEI: 80% IDDM; correlation with diabetes duration
Harano et al. 1978 [76]	53 type 2, 4 type 1, 18 controls	Secretin-pancreozymin test	Diabetes: 69% deficient enzyme output; correlation with diabetes control
Lankisch et al. 1982 [77]	53 type 1	Secretin-pancreozymin test	Diabetes: 43% impaired function
Bretzke et al. 1984 [78]	60 insulin-treated type 2 diabetic patients	Secretin-pancreozymin test	Diabetes: 27% “mild PEI”
El Newihi et al. 1988 [79]	10 type 2 diabetic patients with diarrhea and neuropathy	Secretin and CCK test	Enzyme and bicarbonate reduction in all subjects
Hahn et al. 2008 [9]	33 type 1	Secretin and CCK test	33% mild enzyme reduction

CCK-PZ: cholecystokinin-pancreozymin; PEI: pancreatic elastase-1; IDDM: insulin-dependent diabetes mellitus.

Therefore, a less invasive, cost-effective test was needed to evaluate pancreatic exocrine function in DM. Fecal elastase-1 (FE-1) test measures fecal levels of elastase-1, a proteolytic enzyme produced by pancreatic acinar cells. FE-1 correlates with the output of other pancreatic enzymes, it is highly stable in feces and easy to measure [80]. FE-1 demonstrated good sensitivity and specificity in moderate and severe PEI [81, 82]. Nowadays, therefore, 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 (Table 6b) [4, 5, 9, 83–96].

TABLE 6B. RESULTS OF INDIRECT PANCREATIC FUNCTION TESTS IN PATIENTS WITH DIABETES MELLITUS

Author	Subjects/diabetes type	Methods	Results
Hardt and Kloer 1998 [83]	128 type 1 and 2	Fecal chymotrypsin Fecal elastase 1	45% <6 U/I 46% <200µg/g
Hardt et al. 2000 [5]	39 type 1 77 type 2	Fecal elastase 1	74% <200µg/g 36% <200µg/g
Icks et al. 2001 [84]	112 type 1	Fecal elastase 1	54.5% <200µg/g
Rathmann et al. 2001 [85]	544 type 2	Fecal elastase 1	30.3% <200µg/g
Hardt et al. 2003 [86]	323 type 1 697 type 2	Fecal elastase 1	51% <200µg/g 35% < 200µg/g
Nunes et al. 2003 [87]	42 type 1 and 2	Fecal elastase 1	36% <200µg/g
Cavalot et al. 2004 [88]	66 type 1	Fecal elastase 1	26% <200µg/g
Yilmaztepe et al. 2005 [89]	32 type 2	Fecal elastase 1	28% <200µg/g
Ewald et al. 2007 [90]	546 type 2	Fecal elastase 1	21.1% <100µg/g
Hahn et al. 2008 [9]	33 type 1	Fecal elastase 1	33% <200µg/g
Larger et al. 2012 [91]	195 type 1, 472 type 2	Fecal elastase 1	23% <200µg/g
Vujasinovic et al. 2013 [92]	50 type 1, 100 type 2	Fecal elastase 1	5.4% <200µg/g
Terzin et al. 2014 [93]	101 type 2	Fecal elastase 1	16.8% <200µg/g
Cummings et al. 2015 [4]	288 type 2	Fecal elastase 1	10% <200µg/g
Shivaprasad et al. 2015 [94]	89 type 1, 95 type 2	Fecal elastase 1	31% <200µg/g
Kangrga et al. 2016 [95]	315 type 2	Fecal elastase 1	5.1% <100µg/g and 5.1% <200µg/g
Oscarsson et al. 2017 [96]	10 type 1, 38 type 2	Fecal elastase 1	33% <200µg/g

The prevalence of PEI in both types of diabetes is very heterogenous. However, most of these studies did not exclude cases with previous pancreatic disease, thus leading to a possible bias. In two recent studies, the prevalence of PEI in DM was less frequent than in previous studies, probably because pancreatic (type 3c, according to the new classification of ADA: type 4 [97]) diabetes was excluded [92, 93]. Low FE-1 was measured in only 5.4% of 150 consecutive type 1 and 2 diabetic patients after excluding patients with excessive alcohol consumption, medical history of abdominal surgery, other known reasons for malabsorption, previous pancreatic disease and DM lasting <5 years [92]. In another recent study, PEI was diagnosed with FE-1 measurement in 16.8% of type 2 diabetic patients after excluding

patients with an abnormal pancreatic morphology [93]. Indeed, the prevalence of chronic pancreatic diseases among diabetic patients might be high because recent discussions have suggested that pancreatic diabetes (type 4) has been underestimated in the past and that it might cause about 8% of all diabetes cases [98].

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 [99, 100]. Reduced pancreas size in patients with DM was demonstrated by abdominal US, CT or MRI [101–107]. 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 (Table 2) [99–112].

TABLE 7. THE PREVALENCE OF MORPHOLOGIC CHANGES OF THE EXOCRINE PANCREAS IN DIABETES MELLITUS

Author	Year	Subjects	Methods	Results
Blumenthal HT et al. [108]	1963	3,821 autopsy cases	Morphology	Prevalence of pancreatitis: - In diabetics: 11.2%; - In non-diabetics: 5.3%
Putzke HP et al. [109]	1986	100 diabetic and 100 non-diabetic autopsy cases	Histopathology	Lipomatosis: - In diabetics: 75%; - In controls: 60%
Gilbeau JP et al. [101]	1992	20 type 1, 37 type 2	CT scans	Pronounced lobulation, small size compared to controls
Alzaid A et al. [103]	1993	14 type 1, 43 type 2	Ultrasound	Small size compared to controls; type1<type2<controls
Nakanishi K et al. [104]	1994	36 type 1, 43 type 2	ERCP	Changes like CP: - type 1: 40% - type 2: 9%
Klöppel G et al. [100]	1996	type 1	Histology	Fibrosis, atrophy, fatty infiltration
Foulis AK et al. [99]	1997	type 1	Histology	Fibrosis, atrophy, fatty infiltration

Altobelli E et al. [102]	1998	60 type 1	Ultrasound	Small size compared to controls; dependent on duration
Hardt PD et al. [105]	2002	38 type 1, 118 type 2	ERCP	Changes like CP: type 1>type 2, up to 75%
Williams et al. [111]	2007	12 male patients with type 1 and 12 healthy controls	MRI	Pancreatic volume showed a 48% reduction in long-standing type 1 diabetes as compared with age-matched normal subjects.
Bilgin M et al. [106]	2009	82 type 1 and type 2	MRI/MRCP	Changes like CP
Philippe et al. [107]	2011	24 type 1 and 28 type 2	CT scans	The pancreatic volume, 42cm (25–57cm), was decreased in most patients
Williams et al. [112]	2012	20 male recent-onset type 1 diabetes patients and 24 male healthy controls	MRI	Pancreatic volume is reduced by 26% in type 1 diabetes
Burute N et al. [110]	2014	32 type 2 and 50 normoglycemic individuals	MRI	Patients with type 2 DM had significantly lower pancreatic volume than normoglycemic individuals (p<0.001)

CT: computer tomography; ERCP: endoscopic retrograde cholangiopancreatography; MRI: magnetic resonance imaging; MRCP: magnetic resonance cholangiopancreatography; CP: chronic pancreatitis.

4. DISCUSSION

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

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

A decreased level of PE-1 has been reported to be present in about 50% of patients with type 1 DM and in 30-50% of those with T2DM [6, 7, 113, 114]. However, the relationship between PEI and the level of glycemic control has not yet been accurately determined. As far as we are aware, only one publication and a letter (reporting on 37 and 16 patients, respectively) are available on this topic [12, 13]. The concentration of PE-1 has been demonstrated to correlate with HbA1c, and to be lower in patients with HbA1c >8% than in those with HbA1c ≤8% [12]. On the other hand, no correlation has been revealed between the level of PE-1 and HbA1c, and the level of PE-1 does not differ between patients with HbA1c >8% and those with HbA1c ≤8% [13]. Nevertheless, the ADA recommends that an HbA1c level of 7% should be regarded as the threshold in differentiating between good and poor glycemic control [14]. Furthermore, both studies recruited type 1 DM patients. There is no available data about the relationship between an exocrine pancreatic insufficiency and the level of glycemic control in T2DM. We therefore evaluated the pancreatic exocrine function via the measurement of fecal PE-1 in consecutive T2DM patients, who were grouped as demonstrating either poor (HbA1c ≥7%), or good (HbA1c <7%) glycemic control. Diabetic patients in whom the characteristic morphologic features of CP were revealed, and therefore, type 3c diabetes was diagnosed, were excluded from the study.

PEI was observed in 16.8% (17/101) of the patients with T2DM in our study, which seems to be lower than those in previous reports [6, 7, 113, 115]. However, morphological examinations of the pancreas were performed in all diabetic patients in our study, and CP cases misdiagnosed as T2DM, were excluded. Otherwise, the PEI would have count 20.8% (22/106) of our patients, which is consistent with previous reports [6, 7, 113, 115]. PE-1 concentration < 100 µg/g was recently recommended to indicate PERT in patients with any pancreatic or extrapancreatic disease or condition potentially causing PEI [116]. There were only 3 patients (2.9 % of the recruited cases) with PE-1 < 100 µg/g in our patient cohort, all of

them in Group A. This value is much lower than those reported in previous studies, but corresponds with the result of a recent paper, where marked PEI was remarkably low (2.7%) in diabetic patients [117]. An HbA1c level of 7% was applied in our study as the threshold in differentiating between good and poor glycemic control [14]. Since patients with PE-1 <100 µg/g were having HbA1c level between 7 and 8%, choosing a threshold value of HbA1c level of 8% in differentiating between poor and good glycemic control, the mean PE-1 level would not differ significantly between Group A and B (410.8 ± 150.3 vs. 416.4 ± 172.2 ug/g, $p=0.874$).

There were more cases with a decreased PE-1 level among the patients with poor glycemic control as compared with the patients with good glycemic control. Morphological alterations of the pancreas have been demonstrated in both type 1 and type 2 DM patients [32–35]. Atrophy of the pancreas and an exocrine pancreatic deficiency has been shown to be related events in DM, based on morphological and histological studies [66, 118, 119]. Pancreatic atrophy was frequent in our study, but the diameter of the pancreas body did not differ between patients with poor or good glycemic control (Table 2). Theoretically, the accumulation of fat in acinar cells could cause an exocrine dysfunction in pancreatic steatosis [120], though there is only sparse relevant literature [121, 122]. Furthermore, it has been hypothesized that pancreatic steatosis may play an important role in the pathogenesis of T2DM by leading to damage to the islet cells [123]. Nevertheless, there was no correlation between the occurrence of pancreatic steatosis and a decreased PE-1 level in our study. Overall, the more frequent pancreatic exocrine dysfunction among T2DM patients can not be explained either by pancreatic atrophy or by pancreatic steatosis.

The BMI can not be responsible for the pancreatic exocrine dysfunction in the patients with poor glycemic control, since it did not differ between Groups A and B. The duration of the disease was significantly longer in Group A as compared to Group B, however, no significant correlation was exhibited between the PE-1 level and the disease duration in our patient cohort. Previous studies have yielded contradictory findings on the correlation of the PE-1 level and the duration of the disease in type 1 DM: three papers demonstrated no correlation [13, 113, 115], whereas the duration of DM correlated with the PE-1 level in another two series [6, 12]. Indeed, the 3 patients in Group A with severe exocrine insufficiency had average disease duration of 20 years, which is substantially longer than the disease duration in Group B. Diabetic microangiopathy produces inadequate perfusion and ischemia of the exocrine pancreas, which may lead to pancreatic fibrosis. The longer the duration of diabetes, the bigger the chance is for developing diabetic microangiopathy. Although, the mean age of our patients was significantly higher in Group A as compared to

Group B, no significant correlation was revealed between the PE-1 level and the age of the patients either. The serum level of HbA1c was significantly higher and the PE-1 concentration was significantly lower in Group A as compared to Group B. Acute hyperglycemia has been demonstrated to inhibit both basal and cholecystokinin-stimulated pancreatic enzyme secretion [124]. Poor glycemic control may therefore impair the exocrine pancreatic function via the mechanism of glucotoxicity at the acinar cells. The lack of trophic effect of high local concentrations of insulin may cause impaired secretion of digestive enzymes in the pancreas [124]. Although, serum insulin level was not measured in our study, there were significantly more insulin dependent patients in Group A as compared to Group B (80% vs. 36%), suggesting that the deficit of insulin is more expressed in patients with low PE-1 concentrations. Taken together, hyperglycemia, lack of insulin, the higher age and longer diabetes duration through the development of diabetic neuropathy and pancreatic arteriopathy might all have a contributory role in the development exocrine pancreatic dysfunction in diabetes [7, 115].

An impaired exocrine pancreatic function might influence glucose control, since the exocrine and endocrine pancreata are closely linked both anatomically and physiologically [1, 124]. Since the oral administration of free fatty acids leads to increases in basal and stimulated insulin secretion by beta-cells [114], qualitative fat maldigestion in diabetic patients with PEI might contribute to the poor metabolic control in DM. Furthermore, the absorption of nutriment is unpredictable without adequate enzyme replacement therapy; the glucose-lowering effect of insulin occurs earlier than the glucose-elevating effect of nutriment, thereby leading to hypoglycemia [125, 126]. PERT has been demonstrated to increase glucagon-like peptide-1 (GLP-1), glucosedependent insulintropic polypeptide and insulin secretion and lowers plasma glucose level in patients with CP and PEI [10, 127]. PERT may be beneficial in DM with PEI by increasing meal-stimulated insulin secretion via incretins release. However, 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 [11]. Nevertheless, PERT may be useful in T2DM patients with low PE-1, in whom diabetes could not be adequately controlled.

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

This study demonstrates that NAFLD and NAFPD are already present and PAT volume is increased in patients with NODM. Metformin therapy effectively decreased the amount of fat in the liver but did not affect the amount of fat in the pancreas or PAT volume.

NAFLD is the most common liver disorder worldwide and contributes significantly to overall mortality and to cardiovascular and liver-related mortality in particular. The main risk factor is T2DM; NAFLD can be demonstrated in 64–69% of T2DM patients [16, 21–23]. It is likely that NAFLD is the hepatic manifestation of metabolic syndrome, where insulin resistance is the main risk factor [128]. On the other hand, NAFLD may progress to an inflammatory complication, NASH. The high incidence of NASH in patients with T2DM may lead to further complications, such as liver cirrhosis and hepatocellular carcinoma [30, 31]. Given the expected rise in the prevalence of T2DM, NAFLD is projected to be the principal etiology for liver transplantation within the next decade [21]. Further, NAFLD is believed to be an independent determinant of cardiovascular disease [129].

Metabolic syndrome and obesity are commonly associated with NAFLD. However, the links between NAFLD, insulin resistance, and T2DM are not fully understood [21]. NAFLD can predict the incidence of diabetes independently of traditional risk factors, including obesity, peripheral insulin resistance, and metabolic syndrome [130]. Moreover, diabetes promotes or worsens hepatic steatosis, thus fueling a vicious cycle.

PAT plays a role in myocardial energy metabolism through the connection with the coronary arteries and the myocardium [131, 132]. Increased pericardial fat volume was demonstrated as a risk factor for coronary artery disease [133] and the development of cardiovascular disease in T2DM patients [62]. In the Jackson heart study, PAT was associated with elevated levels of fasting glucose, triglycerides, C-reactive protein, systolic blood pressure, and lower levels of high-density lipoprotein [132]. Moreover, PAT was also linked to metabolic syndrome, hypertension, T2DM, and metabolic syndrome [132]. Further, Iozzo found increased PAT volume in T2DM patients [61]. We demonstrated that PAT volume is already higher in NODM; however, metformin therapy did not affect PAT volume. The latter finding is in line with a previous study, where 24-week-long metformin treatment did not change PAT size [134]. The mechanisms of metformin are complex and are still not fully understood. Metformin works directly or indirectly on the liver to reduce hepatic glucose production, affects the gut to increase glucose utilization and the level of GLP-1, and alters

the microbiome [135]. The GLP-1 receptor is also expressed in the adipose tissue, and GLP-1 promotes adipogenesis by upregulation of adipocyte-specific markers and transcription factors [136]. This may explain why metformin did not decrease PAT volume, although it had a beneficial effect on metabolic parameters.

In our study, a T2DM patient cohort was selected to analyze the effect of insulin resistance on the prevalence of NAFLD. The control subjects did not have DM or any pancreatic, liver, or cardiovascular disease, or history of alcohol consumption, but they were matched for age, sex, BMI, and serum lipids. Therefore, NODM was the only variable in our study that could influence the prevalence of NAFLD. Patients with NODM were enrolled in this prospective study if they had been diagnosed within 1 month, they consumed no alcohol, and their medical records showed no pancreatic, liver, or cardiovascular disease, inherited disorders of fat metabolism, antidiabetic medication or pregnancy, or malignant disease. The aims of the study were (1) to analyze the effect of early phase insulin resistance on the development of NAFLD and NAFPD and (2) to investigate the effect of newly introduced metformin therapy on the degree of fat content in the liver and pancreas and on PAT size.

Sixty-nine percent and 62% of T2DM patients had NAFLD as defined by ultrasound in two previous studies [22, 23], and 87% of the NAFLD cases were confirmed histologically in the latter study. However, these studies featured a cross-sectional design that included diabetic patients with variable disease lengths. We involved NODM patients diagnosed within 1 month before enrollment in our study. Overall, 64.7% of newly diagnosed diabetic patients in our investigation had NAFLD. This means that NAFLD is already present in the early phase of DM. The high prevalence of NAFLD in our study can be explained by the fact that our patients had several risk factors. They were generally overweight and had elevated cholesterol and triglyceride levels. However, BMI, serum cholesterol, and triglyceride levels were also above normal in the control group, and there were no significant differences between the control group and the DM group. In contrast, only 10% of the control subjects had NAFLD. Further, the radiation absorption of the liver was significantly lower, indicating a higher amount of fat in the liver in patients with T2DM compared with the control group. This increased amount of fat, therefore, can be attributed to insulin resistance after excluding other risk factors. It was demonstrated that insulin resistance is already present at least 5 years before overt diabetes in populations with a high prevalence of T2DM [137]. The high prevalence of NAFLD can also be explained by the fact that we defined fatty liver by measuring radiation absorption on CT, which is more sensitive, specific, and operator-independent compared with ultrasound.

NAFPD has been poorly investigated compared with NAFLD, although interest is increasing among researchers. Reports on the relationship between NAFPD and b-cell function are inconsistent. Some studies indicate that pancreatic lipid content is negatively associated with insulin secretion in nondiabetic subjects [45] or individuals with prediabetes [44], whereas others suggest that there is no relationship between b-cell function and pancreatic fat in prediabetic [138] or diabetic subjects [45].

Pancreatic fat content can be studied with multiple diagnostic modalities. A histological examination requires a pancreatic biopsy. However, this is invasive, and there are complications associated with it. Ultrasonography is cheap and easily available, but a relatively insensitive measure of pancreatic fat content. More recently, expensive MRI techniques have been used to assess pancreatic fat deposition. A native CT scan was employed in our study to measure the amount of pancreatic steatosis using radiation absorption correlated to the spleen.

Diabetic patients have been demonstrated to have higher pancreatic fat content as measured by magnetic resonance spectroscopy [45, 49] and dual-echo magnetic resonance chemical shift imaging [139]. In contrast, Saisho et al. found that pancreatic fat content was not significantly increased in T2DM [140]. Overall, 82.3% of NODM patients were diagnosed as having NAFPD based on the diagnostic criteria, whereas NAFPD was only detected in 20% of the control population in our study. Since the control group was matched for age, sex, BMI, and serum lipids with the T2DM group, NAFPD may be a consequence of insulin resistance.

Newly (<1 month) diagnosed T2DM patients were enrolled in the study to assess the effect of metformin on NAFLD and NAFPD. Metformin is the first-line agent for the treatment of diabetes and the most popular antidiabetic agent worldwide. The effects of metformin on NAFLD have been evaluated in several studies, with some of them showing a beneficial effect on aminotransferase levels or liver histological alterations [16, 141–143]. To our knowledge, no study has ever evaluated the effect of metformin on hepatic fat content measured by tissue attenuation during unenhanced CT examination. Four-month-long metformin treatment significantly reduced fat content in the liver in our study. Metformin also improved glycemic control and insulin resistance, as measured by HOMA-IR, and lowered serum cholesterol level, the results of which can partly be attributed to its beneficial effects. However, metformin therapy did not reduce pancreatic fat content and PAT size.

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

Pathophysiology of PEI in diabetes mellitus

The mechanism of PEI in diabetes is multifactorial (Fig. 7).

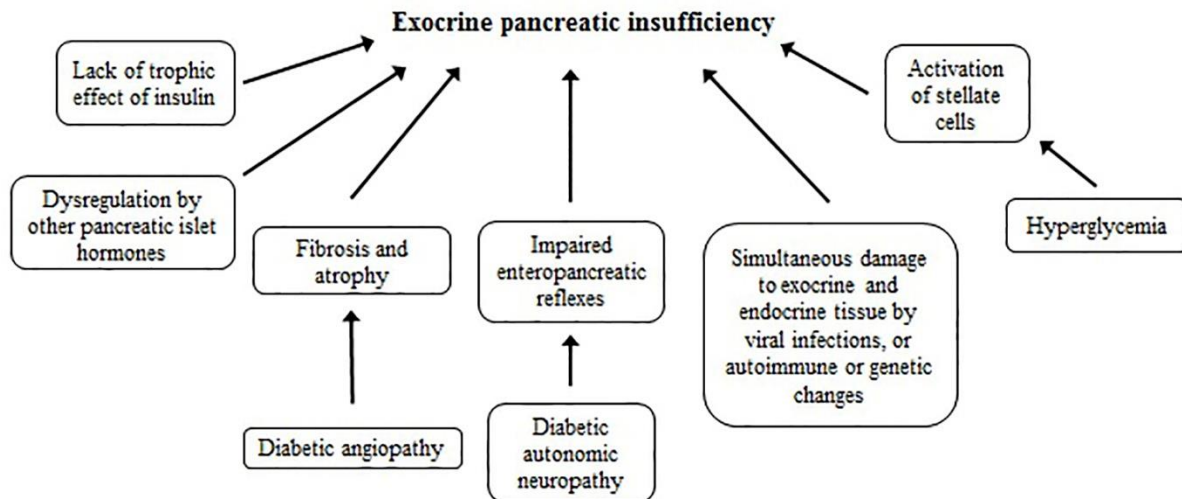


Figure 7. The mechanism of pancreatic exocrine insufficiency in diabetes mellitus

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 [144]. Moreover, decreased pancreatic volume and PEI were shown to correlate in patients with DM [107, 145, 146]. (2) Acute hyperglycemia was demonstrated to inhibit basal and cholecystokinin-stimulated pancreatic enzyme secretion with an insulin-independent mechanism [147]. (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 [148]. (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

[93]. (6) Autonomic neuropathy may give rise to impaired enteropathic reflexes and PEI [91, 149, 150]. Moreover, (7) viral infections [151], (8) autoimmunity [152], or (9) genetic changes, as single-base deletion in the variable number of tandem repeats containing exon 11 of the carboxyl ester lipase gene [153] could increase simultaneous damage to exocrine and endocrine tissue.

The higher prevalence of PEI in type 1 diabetes can be explained by the more severe insulin deficiency, longer disease duration, and higher rate of microvascular complications characterized by type 1 DM.

The correlation between diabetes duration and the prevalence of PEI is contradictory. Previous studies have described an association or at least a weak correlation between low FE-1 level in T2DM and age of onset of diabetes, relatively long diabetes duration, and relatively high HbA1c concentration, suggesting that exocrine dysfunction is a long-term complication of diabetes [86, 154]. However, studies have demonstrated that there is no relationship between fecal elastase concentration and diabetes duration [155]. Otherwise, an inverse correlation was described between diabetes duration and HbA1c levels, and a positive correlation was reported between C-peptide and FE-1 levels [154]. A long-term follow-up study suggested that a mild to moderate exocrine pancreatic insufficiency is due to an early event in the course of DM and does not progress [156].

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 [157]. 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 [4]. As a consequence of malnutrition, PEI is associated with low serum levels of micronutrients, lipid soluble vitamins (vitamins A, D, E, and K), trace elements, albumin, prealbumin and lipoproteins [2, 93, 158–169]. The low level of serum vitamin D leads to osteoporosis and an increased risk of fractures [170]. Protein-energy malnutrition and malabsorption of vitamin D

and other micronutrients may result in a higher risk of infection due to their associated effects on innate and adaptive immune responses [171].

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 [172–174]. In a recent study by Cummings et al. [4], 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. Furthermore, steatorrhea was a poor marker of PEI in diabetes in this study, since only the minority of patients with steatorrhea had a low fecal elastase level. One would logically expect that diabetic patients with PEI experience weight loss, lower body weight and BMI. However, there were no significant differences in BMI between diabetic patients with a decreased or normal PE-1 concentration [4, 93]. Inconsistent with these findings, the size of the pancreas did not correlate with BMI among diabetic patients in another study [101]. Furthermore, PEI detected by low FE-1 concentrations is frequent even in obese diabetic patients [87, 175], and diabetic individuals with excess weight (BMI >25) may be at increased risk for PEI [89].

Diagnosis of PEI

PEI is suggested by clinical symptoms or poor glycemic control despite an adequate diet, antidiabetic therapy and patient adherence [88, 93]. 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 [176–178]. FE-1 concentration correlates with the severity of PEI: a level of less than 200 mg/g stool indicates moderate PEI, while a level of less than 100 mg/g stool indicates severe PEI [179]. FE-1 is not sufficiently sensitive in mild PEI, but if FE-1 level is decreased, there is a strong chance of revealing changes in the pancreatic duct system and steatorrhea [178, 180].

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 [181]. Its accuracy is similar to FE-1 in diagnosing PEI [182].

Coefficient of fat absorption (CFA) is another gold standard test for PEI [183], although it has not been evaluated in DM. During the 72-h stool collection period, the patient consumes 100 g of fat per day. Fat malabsorption is diagnosed at >7 g of fat/100 g of stool/day, with severe steatorrhea at 15 g/day. However, the diet is cumbersome, the 3-day stool collection is inconvenient for both patients and laboratory staff, and therefore CFA is not used in daily clinical practice. It is utilized to evaluate the effectiveness of pancreatic enzyme replacement therapy (PERT) in PEI [184].

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. Three small trials studied the efficacy of PERT in patients with diabetes mellitus secondary to chronic pancreatitis [185, 186]. Treatment with PERT demonstrated a significant reduction in post-prandial plasma glucose and glycosylated hemoglobin at 6 months versus baseline values in patients with diabetes due to tropical calculous pancreatitis [187]. In contrast, PERT did not improve mean glucose values; it produced potentially life-threatening disturbances in glucose control among insulin-dependent diabetic patients due to chronic pancreatitis [188]. However, a recent double-blind, randomized, placebo-controlled trial of PERT in patients with PEI due to chronic pancreatitis demonstrated that the efficacy outcomes and adverse event profile for PERT were comparable between patients with and without diabetes [189]. A larger multicenter, double-blind, randomized, placebo-controlled trial demonstrated that PERT was safe, but has no effect on glycemic control in insulin-treated diabetic patients with FE-1 <100 mg/g [90]. Reduction in mild to moderate hypoglycemic episodes was revealed after 16 weeks of treatment with four capsules of 10 000 FIP units of pancreatin with main meals and two capsules of 10 000 FIP units of pancreatin with snacks, suggesting a more stable control of insulin therapy. However, this study might be criticized. First, patients were selected according to the presence of PEI irrespective of PEI-related symptoms. Second, the applied dose of pancreatin might be low.

Recent guidelines [166, 188–190] 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 [189, 191].

Nutrient-induced glucose-dependent insulintropic polypeptide (GIP) response is diminished in patients with PEI [192]. PERT has been demonstrated to reverse an impaired GIP response and therefore to restore the incretin effect of fat [192]. 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 [193]. Furthermore, patients with diabetes mellitus have an increased risk of bone fractures [194]. 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 [90].

However, there are several limitations to this systematic review. Firstly, the prevalence of PEI in both types of diabetes is very heterogenous, ranging between 5.1 and 80%. Secondly, studies applied the gold standard direct pancreatic function test in the measurement of PEI are limited to a small number of patients because of the invasive nature of the test. Thirdly, most of these studies did not exclude cases with previous pancreatic disease, thus leading to a possible bias. Fourth, PEI seems to be frequent in DM, data on the occurrence of the symptoms of PEI in diabetes are limited. Furthermore, only a very limited number of publications have investigated the effectiveness of PERT in PEI associated with diabetes, and the results are contradictory.

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.

5. 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.

6. SUMMARY

BACKGROUND: 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. Abdominal symptoms such as nausea, bloating, diarrhea, steatorrhea, and weight loss can often occur in diabetic patients. These symptoms may be attributed to the side-effects of the metformin they are taking, the autonomic neuropathy on bowel function, small bowel bacterial overgrowth, celiac disease, or pancreatic exocrine insufficiency (PEI). Impairments of the exocrine pancreatic function seem to be a frequent complication of diabetes mellitus (DM); however, they are largely overlooked. Greater knowledge and awareness are required in testing and diagnosing this condition.

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]). Several studies have suggested that insulin resistance is associated with pancreatic fat accumulation, nonalcoholic steatohepatitis (NASH), and pre-DM. Besides epicardial adipose tissue, PAT is another risk factor for the development of cardiovascular disease in type 2 DM (T2DM) patients. Our aims were (i) to assess the possible relationship between T2DM with poor glycemic control ($\text{HbA1c} \geq 7.0\%$), 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 diabetes mellitus (NODM); (iii) to provide an overview of the current concepts of PEI in diabetes mellitus by performing a systematic review.

PATIENTS AND METHODS: For these purposes we carried out two different clinical studies and one systematic review. In our first study, patients with type 2 DM treated in our clinic were prospectively recruited into In our first study. Pancreatic diabetes was excluded. Cases with $\text{HbA1c} \geq 7\%$ formed Group A ($n=59$), and with $\text{HbA1c} < 7\%$ Group B ($n=42$). The fecal level of pancreatic elastase-1 (PE-1) was measured and morphological examinations of the pancreas were performed. In the second study, seventeen patients with NODM and 10 subjects used as a control group were involved in the study. Computed tomography (CT) and laboratory tests were performed before the beginning of metformin therapy and 4 months afterward. PAT and the amount of fat in the pancreas and liver were determined by X-ray attenuation during unenhanced CT examination and compared with the values for the control subjects. In our third study, which was performed in 2018, 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 III/C diabetes 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 judgement, then a committee of three other researchers was invited to draw a conclusion.

RESULTS AND CONCLUSIONS: the first study has demonstrated that the PE-1 level was significantly lower in Group A than in Group B ($385.9 \pm 171.1 \mu\text{g/g}$, vs. $454.6 \pm 147.3 \mu\text{g/g}$, $p=0.038$). 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 ($r=0.010$, $p=0.921$), BMI ($r=0.203$, $p=0.059$), or pancreatic steatosis ($r=0.117$, $p=0.244$). The size of the pancreas did not differ significantly between Groups A and B. PEI demonstrated by fecal PE-1 determination is more frequent in T2DM patients with poor glycemic control. The impaired exocrine pancreatic function cannot be explained by an alteration in the size of the pancreas or by pancreatic steatosis. The second study has suggested that metabolic parameters improved significantly after metformin therapy. NAFLD was diagnosed in 64.7% of the patients with NODM and in 10% of the control subjects. The radiation absorption of the liver was significantly lower in the patients with NODM compared with the control group and significantly higher after metformin therapy compared with the baseline values. Only six patients (35.3%) had NAFLD after metformin therapy. NAFLD was diagnosed in 82.3% of the patients with NODM and in 20% of the control subjects. The radiation absorption of the pancreas was significantly lower in the patients with NODM compared with the control group but did not change significantly after treatment. PAT size was significantly larger in the patients with NODM and did not change significantly after metformin treatment. NAFLD, NAFLD, and increased PAT were detected in the majority of patients with NODM. 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. The prevalence and symptoms of PEI in diabetes mellitus, the pathomechanism, and difficulties of diagnosis and therapy of PEI are summarized in our systematic review. The currently available evidence is limited to answering the question of whether pancreatic enzyme replacement therapy (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.

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9. ANNEXES

Annex I.



Contents lists available at ScienceDirect

Pancreatology

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Original article

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



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ABSTRACT

Objectives: To evaluate the relationship between exocrine pancreatic insufficiency and the level of glycemic control in diabetes (DM).

Methods: Patients with type 2 DM treated in our clinic were prospectively recruited into the study. Pancreatic diabetes was excluded. Cases with HbA1c $\geq 7\%$ formed Group A ($n = 59$), and with HbA1c $< 7\%$ Group B ($n = 42$). The fecal level of pancreatic elastase (PE-1) was measured and morphological examinations of the pancreas were performed.

Results: The PE-1 level was significantly lower in Group A than in Group B ($385.9 \pm 171.1 \mu\text{g/g}$, vs. $454.6 \pm 147.3 \mu\text{g/g}$, $p = 0.038$). 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 ($r = 0.010$, $p = 0.921$), BMI ($r = 0.203$, $p = 0.059$), or pancreatic steatosis ($r = 0.117$, $p = 0.244$). The size of the pancreas did not differ significantly between Groups A and B.

Conclusions: An exocrine pancreatic insufficiency demonstrated by fecal PE-1 determination is more frequent in type 2 DM patients with poor glycemic control. The impaired exocrine pancreatic function cannot be explained by an alteration in the size of the pancreas or by pancreatic steatosis.

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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 [1]. An exocrine pancreatic insufficiency 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

(DM) cases [2,3]. Steatorrhea was observed in about 60% of patients with diabetes mellitus and fecal elastase $< 100 \mu\text{g/g}$, indicating relevant, severe damage of the exocrine function [4]. Pancreatic enzyme replacement therapy (PERT) in patients with diabetes mellitus and exocrine pancreatic insufficiency may improve glucose metabolism, nutritional parameters and incretin response [5]. However, the significance of an exocrine dysfunction in DM has recently been questioned [6], and no beneficial effect of PERT on the glucose metabolism was observed in patients with insulin treatment for diabetes mellitus, although a reduction in mild and moderate hypoglycemia was demonstrated [7].

Besides the exocrine function, the pancreas morphology has been revealed by ultrasonography (US), computer tomography (CT), endoscopic retrograde pancreatography or autopsy to be altered in many patients with DM [8–11]. Nonalcoholic fatty pancreas disease (NAFPD), defined as pancreatic fat accumulation, has been demonstrated to be associated with obesity, metabolic

Abbreviations: DM, diabetes mellitus; HbA1c, glycated hemoglobin; PE-1, pancreatic elastase; US, ultrasonography; CT, computer tomography; NAFPD, nonalcoholic fatty pancreas disease; ADA, American Diabetes Association; CP, chronic pancreatitis; BMI, body mass index; OR, odds ratio; PERT, pancreatic enzyme replacement therapy.

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syndrome and type 2 DM [12–14], and it has been suggested that NAFPD may cause an endocrine and exocrine pancreatic dysfunction [12].

An exocrine pancreatic insufficiency has not been accurately determined to date in relation to the level of glycemic control. The level of pancreatic elastase-1 (PE-1) has been reported to be lower [15] or not to differ [16] 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% [17].

The aim of the present prospective study was an evaluation of the possible relationship between type 2 DM with poor glycemic control (HbA1c ≥7.0%), an exocrine pancreatic insufficiency and alterations in pancreatic morphology.

Patients and methods

Patients

Consecutive patients with type 2 DM 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 (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) [17]. Chronic pancreatitis (CP) was diagnosed only when both the morphological and functional diagnostic criteria were fulfilled [18]. Cases with type 3c DM, i.e. diabetes secondary to exocrine pancreatic diseases, were excluded. The patients were not operated on the pancreas.

All patients provided their written informed consent to participation. The study protocol was in full accordance with the most recent revisions of the Helsinki Declaration and was approved by the ethics committee at the University of Szeged (200913).

Methods

Fecal PE-1 was determined through the use of monoclonal antibodies (ScheBo Biotech AG, Giessen, Germany). PE-1 concentrations >200 µg/g in the stools indicated a normal exocrine pancreatic function, concentrations between 100 and 200 µg/g indicated a mild exocrine pancreatic insufficiency, and concentrations <100 µg/g denoted a severe exocrine pancreatic insufficiency [19].

Abdominal US and CT were performed to detect the characteristic morphological features of CP. US was applied for the detection of pancreatic steatosis. The liver, or the kidney if the liver was hyperechogenic, was used as reference point; echogenicity of the pancreas higher than that of the liver or kidney parenchyma was an indicator of pancreatic steatosis [20]. The thickness of the pancreas was measured by US just across the spine [8,21]. All US examinations were carried out by the same gastroenterologist, who was blind to the other results on the patients.

Statistical analysis

The experimental data were analyzed statistically with the independent-samples *t*-test, the Mann–Whitney *U* test, the chi-square test. The linear association between two variables was

compared by Pearson-correlation analysis. *p* values <0.05 were accepted as being statistically significant. Statistical data are expressed as means ± standard deviation (SPSS 13.0 for Windows).

Results

A total of 106 type 2 diabetic patients were recruited between April 1, 2012 and June 30, 2013. Five patients were excluded, because morphological examinations revealed the characteristic features (calcifications and dilation of the main pancreatic duct) of advanced CP, all of them with poor glycemic control and with a decreased PE-1 concentration. Consequently, 101 type 2 diabetic patients were included into the study: 59 (25 male, 34 female, mean age: 63.6 ± 10.4 y, range: 42–89 y) in Group A and 42 (22 male, 20 female, mean age: 57.1 ± 11.2 y, range: 30–83 y) in Group B. Two 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 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 (Table 1).

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 exocrine pancreatic insufficiency. In Group B, the PE-1 concentration was normal in 39 subjects (92.9%), while 3 (7.1%) displayed a mild exocrine pancreatic insufficiency, 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 decreased in overall 16.8% of the cases. There were no significant differences in BMI between the patients with a decreased or a normal PE-1 concentration within Group A or B (Table 1).

The PE-1 level was significantly lower in Group A than in Group B (385.9 ± 171.1 µg/g 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) (Fig. 1).

US revealed pancreatic steatosis in 35 patients: 19 in Group A and 16 in Group B. Pancreatic steatosis was less frequent in Group A (32.2%) than in Group B (38.1%) (OR = 0.77), but it was more common in patients with an abnormal PE-1 concentration as compared with those with a normal PE-1 concentration (OR = 1.4)

Table 1
Clinical characteristics of the patients.

	Group A	Group B	<i>p</i>
No. of patients	59	42	—
Male/female	25/34	22/20	—
Mean age (y)	63.6 ± 10.4	57.1 ± 11.2	<i>p</i> < 0.008
Duration of DM (mean ± SD) (y)	13.2 ± 8.9	8.3 ± 7.9	<i>p</i> < 0.006
BMI (mean ± SD)	29.74 ± 5.65	28.43 ± 5.23	<i>p</i> = 0.278
Mean BMI of patients with decreased PE-1 (mean ± SD)	29.99 ± 4.58	32.29 ± 5.29	<i>p</i> = 0.658
Treatment of diabetes (oral antidiabetics/insulin/diet only)	12/47/0	23/15/4	—
Fasting plasma glucose (mean ± SD) (mmol/l)	10.56 ± 3.64	7.03 ± 1.65	<i>p</i> < 0.001
Mean HbA1c (range) (%)	8.3 (7–11.6)	6.2 (5–6.9)	<i>p</i> < 0.001

Data are reported as means with ranges in parentheses or as means ± SD.

DM: diabetes mellitus.

BMI: body mass index.

PE-1: pancreatic elastase-1.

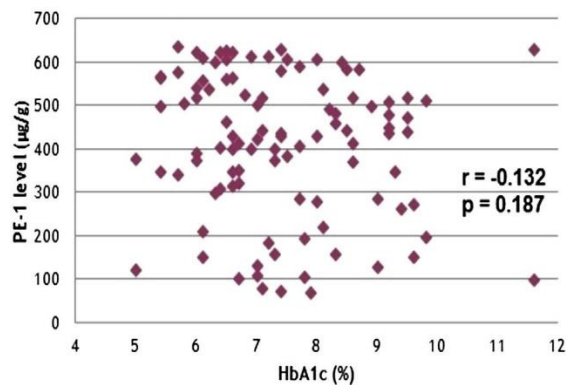


Fig. 1. Correlation between PE-1 level and HbA1c. PE-1: pancreatic elastase, HbA1c: glycated hemoglobin, *r*: correlation coefficient, *p*: the attained level of significance.

(Table 2). The PE-1 level was not correlated with the presence of pancreatic steatosis ($r = 0.117$, $p = 0.244$). There were no other morphological alterations of the pancreas in our patients.

The thickness of the pancreas did not differ significantly between Groups A and B, and there was no correlation between the PE-1 concentration and the size of the pancreas ($r = -0.061$, $p = 0.670$).

The pancreatic morphology could not be evaluated in five subjects due to no compliance.

Discussion

The present study has demonstrated that an exocrine pancreatic insufficiency revealed by fecal PE-1 determination is more frequent in type 2 DM patients with poor glycemic control. The impaired exocrine pancreatic function cannot be explained by an alteration in the size of the pancreas or by pancreatic steatosis.

A decreased level of PE-1 has been reported to be present in about 50% of patients with type 1 DM and in 30–50% of those with type 2 DM [2, 3, 22, 23]. However, the relationship between an exocrine pancreatic insufficiency and the level of glycemic control has not yet been accurately determined. As far as we are aware, only one publication and a letter (reporting on 37 and 16 patients, respectively) are available on this topic [15,16]. The concentration of PE-1 has been demonstrated to correlate with HbA1c, and to be lower in patients with HbA1c >8% than in those with HbA1c ≤8% [15]. On the other hand, no correlation has been revealed between the level of PE-1 and HbA1c, and the level of PE-1 does not differ between patients with HbA1c >8% and those with HbA1c ≤8% [16]. Nevertheless, the ADA recommends that an HbA1c level of 7% should be regarded as the threshold in differentiating between good and poor glycemic control [17]. Furthermore, both studies recruited type 1 DM patients. There is no available data about the relationship between an exocrine pancreatic insufficiency and the level of glycemic control in type 2 DM. We therefore evaluated

the pancreatic exocrine function via the measurement of fecal PE-1 in consecutive type 2 DM patients, who were grouped as demonstrating either poor (HbA1c ≥7%), or good (HbA1c <7%) glycemic control. Diabetic patients in whom the characteristic morphologic features of chronic pancreatitis were revealed, and therefore, type 3c diabetes was diagnosed, were excluded from the study.

An exocrine pancreatic insufficiency was observed in 16.8% (17/101) of the patients with type 2 DM in our study, which seems to be lower than those in previous reports [2,3,22,24]. However, morphological examinations of the pancreas were performed in all diabetic patients in our study, except for five subjects due to no compliance, and CP cases misdiagnosed as type 2 DM, were excluded. Otherwise, the exocrine pancreatic insufficiency would have count 20.8% (22/106) of our patients, which is consistent with previous reports [2,3,22,24]. PE-1 concentration <100 µg/g was recently recommended to indicate PERT in patients with any pancreatic or extrapancreatic disease or condition potentially causing pancreatic exocrine insufficiency [25]. There were only 3 patients (2.9% of the recruited cases) with PE-1 < 100 µg/g in our patient cohort, all of them in Group A. This value is much lower than those reported in previous studies, but corresponds with the result of a recent paper, where marked pancreatic exocrine insufficiency was remarkably low (2.7%) in diabetic patients [26]. An HbA1c level of 7% was applied in our study as the threshold in differentiating between good and poor glycemic control [17]. Since patients with PE-1 < 100 µg/g were having HbA1c level between 7 and 8%, choosing a threshold value of HbA1c level of 8% in differentiating between poor and good glycemic control, the mean PE-1 level would not differ significantly between Group A and B (410.8 ± 150.3 µg/g vs. 416.4 ± 172.2 µg/g, $p = 0.874$).

There were more cases with a decreased PE-1 level among the patients with poor glycemic control as compared with the patients with good glycemic control. Morphological alterations of the pancreas have been demonstrated in both type 1 and type 2 DM patients [8–11]. Atrophy of the pancreas and an exocrine pancreatic deficiency has been shown to be related events in DM, based on morphological and histological studies [21,27,28]. Pancreatic atrophy was frequent in our study, but the diameter of the pancreas body did not differ between patients with poor or good glycemic control (Table 2.). Theoretically, the accumulation of fat in acinar cells could cause an exocrine dysfunction in pancreatic steatosis [12], though there is only sparse relevant literature [29,30]. Furthermore, it has been hypothesized that pancreatic steatosis may play an important role in the pathogenesis of type 2 DM by leading to damage to the islet cells [31]. Nevertheless, there was no correlation between the occurrence of pancreatic steatosis and a decreased PE-1 level in our study. Overall, the more frequent pancreatic exocrine dysfunction among type 2 DM patients cannot be explained either by pancreatic atrophy or by pancreatic steatosis.

The BMI cannot be responsible for the pancreatic exocrine dysfunction in the patients with poor glycemic control, since it did not differ between Groups A and B. The duration of the disease was significantly longer in Group A as compared to Group B, however, no significant correlation was exhibited between the PE-1 level and the disease duration in our patient cohort. Previous studies have yielded contradictory findings on the correlation of the PE-1 level and the duration of the disease in type 1 DM: three papers demonstrated no correlation [16,22,24], whereas the duration of DM correlated with the PE-1 level in another two series [2, 15]. Indeed, the 3 patients in Group A with severe exocrine insufficiency had average disease duration of 20 years, which is substantially longer than the disease duration in Group B. Diabetic microangiopathy produces inadequate perfusion and ischemia of the exocrine pancreas, which may lead to pancreatic fibrosis. The

Table 2
Morphological findings of the pancreas.

	Group A (59 cases)	Group B (42 cases)	<i>p</i>
Morphology of pancreas			
Normal	39 (66.1%)	20 (47.6%)	$p = 0.063$
Steatosis	19 (32.2%)	16 (38.1%)	$p = 0.54$
Malignancy	1 (1.7%)	1 (2.4%)	$p = 0.809$
Thickness (mm)	14.8 ± 5.8	12.6 ± 3.7	$p = 0.112$
No data (noncompliance)	0 (0%)	5 (11.9%)	

longer the duration of diabetes, the bigger the chance is for developing diabetic microangiopathy. Although, the mean age of our patients was significantly higher in Group A as compared to Group B, no significant correlation was revealed between the PE-1 level and the age of the patients either. The serum level of HbA1c was significantly higher and the PE-1 concentration was significantly lower in Group A as compared to Group B. Acute hyperglycemia has been demonstrated to inhibit both basal and cholecystokinin-stimulated pancreatic enzyme secretion [32]. Poor glycemic control may therefore impair the exocrine pancreatic function via the mechanism of glucotoxicity at the acinar cells. The lack of trophic effect of high local concentrations of insulin may cause impaired secretion of digestive enzymes in the pancreas [32]. Although, serum insulin level was not measured in our study, there were significantly more insulin dependent patients in Group A as compared to Group B (80% vs. 36%), suggesting that the deficit of insulin is more expressed in patients with low PE-1 concentrations. Taken together, hyperglycemia, lack of insulin, the higher age and longer diabetes duration through the development of diabetic neuropathy and pancreatic arteriopathy might all have a contributory role in the development exocrine pancreatic dysfunction in diabetes [3,24].

An impaired exocrine pancreatic function might influence glucose control, since the exocrine and endocrine pancreata are closely linked both anatomically and physiologically [1,32]. Since the oral administration of free fatty acids leads to increases in basal and stimulated insulin secretion by beta-cells [23], qualitative fat maldigestion in diabetic patients with an exocrine pancreatic insufficiency might contribute to the poor metabolic control in DM. Furthermore, the absorption of nutrients is unpredictable without adequate enzyme replacement therapy; the glucose-lowering effect of insulin occurs earlier than the glucose-elevating effect of nutrients, thereby leading to hypoglycemia [33,34]. PERT has been demonstrated to increase glucagon-like peptide-1, glucosedependent insulinotropic polypeptide and insulin secretion and lowers plasma glucose level in patients with chronic pancreatitis and pancreatic exocrine insufficiency [5,35]. PERT may be beneficial in DM with pancreatic exocrine insufficiency by increasing meal-stimulated insulin secretion via incretins release. However, no beneficial effect of PERT on the glucose metabolism was observed in patients with insulin treatment for diabetes mellitus, although a reduction in mild and moderate hypoglycemia was demonstrated [7]. Nevertheless, PERT may be useful in type 2 DM patients with low PE-1, in whom diabetes could not be adequately controlled.

In summary, an exocrine pancreatic insufficiency is more frequent in type 2 DM patients with poor glycemic control. The impaired exocrine pancreatic function cannot be explained by an alteration in the size of the pancreas or by pancreatic steatosis. Further studies on a larger cohort of patients are needed to confirm our results and to elucidate the pathomechanism and the clinical significance of this finding.

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Annex II.

In New-Onset Diabetes Mellitus, Metformin Reduces Fat Accumulation in the Liver, But Not in the Pancreas or Pericardium

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 András Nagy, MD,² András Palkó, MD, DSc,² and László Czákó, MD, DSc¹

Abstract

Background: Nonalcoholic fatty pancreas and liver disease (NAFPD and NAFLD) and pericardial adipose tissue (PAT) are often associated with type 2 diabetes mellitus (T2DM). Our aim was to evaluate the incidence rate of NAFLD and NAFPD, PAT size, and the effect of metformin treatment on NAFLD, NAFPD, and PAT in new-onset T2DM (NODM).

Methods: Seventeen patients with NODM and 10 subjects used as a control group were involved in the study. Computed tomography (CT) and laboratory tests were performed before the beginning of metformin therapy and 4 months afterward. PAT and the amount of fat in the pancreas and liver were determined by X-ray attenuation during unenhanced CT examination and compared with the values for the control subjects.

Results: Metabolic parameters improved significantly after metformin therapy. NAFLD was diagnosed in 64.7% of the patients with NODM and in 10% of the control subjects. The radiation absorption of the liver was significantly lower in the patients with NODM compared with the control group and significantly higher after metformin therapy compared with the baseline values. Only six patients (35.3%) had NAFLD after metformin therapy. NAFPD was diagnosed in 82.3% of the patients with NODM and in 20% of the control subjects. The radiation absorption of the pancreas was significantly lower in the patients with NODM compared with the control group but did not change significantly after treatment. PAT size was significantly larger in the patients with NODM and did not change significantly after metformin treatment.

Conclusions: NAFLD, NAFPD, and increased PAT were detected in the majority of patients with NODM. 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.

Keywords: pericardial adipose tissue, type 2 diabetes mellitus, metformin, nonalcoholic fatty pancreas disease, nonalcoholic fatty liver disease

Introduction

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]).¹ 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.^{2–6} There is a high prevalence of NAFLD

in patients with type 2 diabetes mellitus (T2DM) (64%–69%)^{2,7–10} and dyslipidemia (20%–81%).^{11,12}

Obesity, T2DM, and dyslipidemia are risk factors for the development of NAFLD.^{8,9,13,14} The incidence rate of pre-diabetes mellitus (DM) is as high as 93.3% in NAFLD, so pre-DM is a more important predictor of NAFLD than metabolic syndrome.¹⁵ Moreover, it seems that the male gender is presumably a further risk factor for NAFLD.² If left untreated, NAFLD may progress through steatohepatitis to cirrhosis and hepatocellular carcinoma.^{16,17}

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NAFPD is a less well-studied phenomenon. Fatty pancreas is a common ultrasound finding with increased echogenicity of the parenchyma due to fat accumulation.¹⁸ Previous studies have suggested a 16%–35% prevalence of fatty pancreas in the general population.^{19,20} It seems that age,²¹ obesity, hyperglycemia, and dyslipidemia are risk factors for NAFPD.^{20,22–24} Also, NAFPD may increase the risk for the development of metabolic syndrome by causing inflammation,²⁵ impaired pancreatic beta cell function, and finally hyperglycemia.²⁶ This relationship may explain the presence of T2DM²⁷ in 6.9%–12.6% of patients with pancreatic steatosis.^{19,20} Several studies have suggested that insulin resistance is associated with pancreatic fat accumulation,^{22–24,28,29} nonalcoholic steatohepatitis (NASH),^{22,30} and pre-DM.²³

Higher pancreatic triglyceride content in obesity can be detected by proton magnetic resonance spectroscopy,³¹ computed tomography (CT),^{32,33} or magnetic resonance imaging (MRI)³⁴ even before the development of T2DM.³¹ It has been demonstrated that obesity may lead to pancreatic ductal adenocarcinoma through pancreatic steatosis.^{25,35,36} Evidence suggests that pancreatic steatosis plays a role in T2DM, pancreatic exocrine dysfunction, acute pancreatitis,^{37,38} pancreatic cancer, and the formation of pancreatic fistulas after pancreatic surgery.³⁹

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.⁴⁰ The elevation of liver transaminases may suggest the presence of NAFLD or NASH. Ultrasonography 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.⁴¹ 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 ultrasonography is more difficult, and the sensitivity and specificity of ultrasonography in detecting NAFPD are hampered by obesity and bloating.

Further, in prediabetic and T2DM patients, the amount of PAT is significantly higher compared with that in normoglycemic patients.^{42,43} Previous reviews have demonstrated that, besides epicardial adipose tissue, PAT is another risk factor for the development of cardiovascular disease in T2DM patients.⁴⁴

The aim of this study was to evaluate the incidence rate of NAFLD and NAFPD, PAT size, and the effect of metformin treatment on NAFLD, NAFPD, and PAT in new-onset T2DM (NODM) by measuring tissue attenuation during unenhanced CT examination.

Subjects and Methods

Seventeen patients with NODM (male: 8; female: 9; mean age: 55.3 ± 10.8 years; body mass index [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 (Table 1).

The diagnosis of T2DM was made in accordance with the American Diabetes Association criteria.⁴⁵ NODM is defined

TABLE 1. AGE, BODY MASS INDEX, CHOLESTEROL, AND TRIGLYCERIDE LEVELS IN PATIENTS WITH NEW-ONSET TYPE 2 DIABETES MELLITUS AND HEALTHY CONTROL SUBJECTS AT BASELINE

Parameters	Control (male: 6; female: 4)	NODM (male: 8; female: 9)	P
Age (years)	53.9 ± 13.8	55.3 ± 10.8	0.521
BMI (kg/m ²)	30.0 ± 4.7	31.8 ± 5.1	0.437
Serum cholesterol (mM)	5.1 ± 0.9	5.7 ± 1.9	0.187
Serum triglyceride (mg/dL)	3.3 ± 2.7	3.1 ± 1.8	0.830

Data expressed as mean values \pm SD.

BMI, body mass index; NODM, new-onset type 2 diabetes mellitus; SD, standard deviation.

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 glycosylated hemoglobin [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]) (Fig. 1). 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.⁴⁶ 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 .^{47,48}

All the participants provided written informed consent. The study protocol was in full accordance with the most recent revisions of the Helsinki Declaration and was approved by the ethics committee at the University of Szeged.

Statistical analysis

Continuous measures are summarized and presented as means and standard deviations. Categorical data are presented as percentages. The two-sample *t*-test and paired-samples *t*-test were used to determine differences between continuous parameters. Non-normally distributed data were log transformed. Data were processed with SPSS 22.0 (Armonk, NY), and a level of $P < 0.05$ was considered statistically significant.

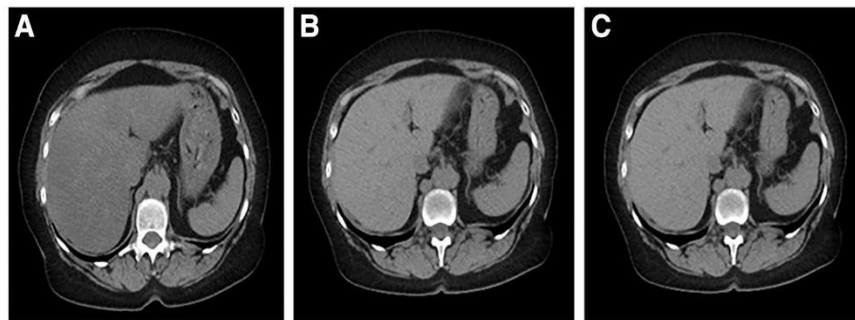


FIG. 1. Typical plain CT images in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. The density of the liver was diffusely decreased in new-onset type 2 diabetic patients (A) as compared with control (C). Metformin therapy increased liver density in the diabetic patients (B) like the density in the control liver (C). CT, computed tomography.

Results

Fasting blood glucose, HbA1c, serum insulin and cholesterol, and HOMA-IR decreased significantly after metformin therapy compared with the baseline values (Table 2). 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. NODM patients with and without NAFLD were compared (Table 3). 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 (Table 3).

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 ± 8.3 HU [$P=0.001$]) and significantly increased after metformin therapy compared with

the baseline values (32.3 ± 17.7 vs. 47.3 ± 12.1 HU [$P=0.026$]) (Fig. 2). Only six patients (35.3%) had NAFLD after the 4-month metformin therapy according to the diagnostic criteria.

NAFLD 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 NAFLD 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 [$P=0.178$]) (Fig. 3).

PAT size was significantly larger in the patients with NODM compared with the control group (2143.1 ± 1036 vs. 1223.9 ± 312.9 mm² [$P=0.008$]) and did not change significantly after the metformin treatment (2143.1 ± 1036 vs. 2048.2 ± 997 mm² [$P=0.798$]) (Fig. 4).

Discussion and Conclusions

This study demonstrates that NAFLD and NAFLD are already present and PAT volume is increased in patients with NODM. Metformin therapy effectively decreased the amount of fat in the liver but did not affect the amount of fat in the pancreas or PAT volume.

NAFLD is the most common liver disorder worldwide and contributes significantly to overall mortality and to cardiovascular and liver-related mortality in particular. The main risk factor is T2DM; NAFLD can be demonstrated in 64%–69% of T2DM patients.^{2,7–9} It is likely that NAFLD is the hepatic manifestation of metabolic syndrome, where insulin resistance is the main risk factor.⁴⁹ On the other hand, NAFLD may progress to an inflammatory complication, NASH. The high incidence of NASH in patients with T2DM may lead to further complications, such as liver cirrhosis and hepatocellular carcinoma.^{16,17} Given the expected rise in the prevalence of T2DM, NAFLD is projected to be the principal etiology for liver transplantation within the next decade.⁷ Further, NAFLD is believed to be an independent determinant of cardiovascular disease.⁵⁰

Metabolic syndrome and obesity are commonly associated with NAFLD. However, the links between NAFLD,

TABLE 2. CHANGES OF LABORATORY TEST RESULTS AND BODY MASS INDEX FROM BASELINE VALUES TO 4 MONTHS AFTERWARD DURING METFORMIN THERAPY IN PATIENTS WITH NEW-ONSET TYPE 2 DIABETES MELLITUS

Parameters	Baseline values (male: 8; female: 9)	4 months afterward (male: 8; female: 9)	P
Fasting blood glucose (mM)	12.9 ± 4.8	7.0 ± 1.4	0.001
HbA1c (%)	9.6 ± 2.8	6.7 ± 0.8	<0.001
Serum insulin (mU/L)	33.1 ± 18.3	22.2 ± 11.5	0.003
HOMA-IR (molar units)	16.6 ± 9.0	7.1 ± 3.1	<0.001
Serum cholesterol (mM)	4.8 ± 1.0	4.4 ± 1.0	0.016
Serum triglyceride (mg/dL)	2.9 ± 1.1	2.4 ± 1.3	0.299
BMI (kg/m ²)	31.8 ± 5.1	31.6 ± 4.4	0.757

Data expressed as mean values \pm SD.

HbA1c, glycosylated hemoglobin; HOMA-IR, homeostatic model assessment-estimated insulin resistance. Significance level $p<0.05$ for figures that are **bold italic**.

TABLE 3. AMOUNT OF FAT IN THE LIVER, CHOLESTEROL, TRIGLYCERIDE, BODY MASS INDEX, FASTING BLOOD GLUCOSE, HbA1c, AND LIVER ENZYME VALUES IN NEW-ONSET TYPE 2 DIABETES MELLITUS PATIENTS WITH AND WITHOUT NONALCOHOLIC FATTY LIVER DISEASE AT BASELINE

Parameters	Patients with NAFLD (male: 6; female: 5)	Patients without NAFLD (male: 2; female: 4)	P
The amount of fat in the liver (HU)	25.2 ± 12.7	55.6 ± 9.3	0.002
Serum cholesterol (mM)	5.5 ± 1.1	5.8 ± 0.3	0.002
Serum triglyceride (mg/dL)	2.7 ± 1.4	2.9 ± 1.5	0.776
BMI (kg/m ²)	31.6 ± 5.2	29.2 ± 5.6	0.233
Aspartate aminotransferase (U/L)	20.6 ± 5.5	16.5 ± 3.0	0.123
Alanine aminotransferase (U/L)	24.9 ± 8.6	19.5 ± 6.5	0.151
Alkaline phosphatase (U/L)	76.8 ± 18.0	83.5 ± 24.4	0.717
Gamma glutamyltransferase (U/L)	43.9 ± 15.2	46.3 ± 25.6	0.901
Total bilirubin (μM)	8.1 ± 1.7	5.9 ± 2.5	0.245
Fasting blood glucose (mM)	13.9 ± 5.9	12.3 ± 9.2	0.882
HbA1c (%)	9.4 ± 2.1	9.2 ± 2.8	0.924
HOMA-IR	17.8 ± 8.3	11.4 ± 9.5	0.368

Data expressed as mean value ± SD.

HU, Hounsfield unit; NAFLD, nonalcoholic fatty liver disease. Significance level $p < 0.05$ for figures that are **bold italic**.

insulin resistance, and T2DM are not fully understood.⁷ NAFLD can predict the incidence of diabetes independently of traditional risk factors, including obesity, peripheral insulin resistance, and metabolic syndrome.⁵¹ Moreover, diabetes promotes or worsens hepatic steatosis, thus fueling a vicious cycle.

PAT plays a role in myocardial energy metabolism through the connection with the coronary arteries and the myocardium.^{52,53} Increased pericardial fat volume was demonstrated as a risk factor for coronary artery disease⁵⁴ and the development of cardiovascular disease in T2DM patients.⁴⁴ In the Jackson heart study, PAT was associated with elevated levels of fasting glucose, triglycerides, C-reactive protein, systolic blood pressure, and lower levels of high-density lipoprotein.⁵³ Moreover, PAT was also linked to metabolic syndrome, hypertension, T2DM, and metabolic syndrome.⁵³ Further, Lozzo found increased PAT volume in T2DM patients.⁴³ We demonstrated that PAT volume is already higher in NODM; however, metformin therapy did not af-

fect PAT volume. The latter finding is in line with a previous study, where 24-week-long metformin treatment did not change PAT size.⁵⁵ The mechanisms of metformin are complex and are still not fully understood. Metformin works directly or indirectly on the liver to reduce hepatic glucose production, affects the gut to increase glucose utilization and the level of glucagon-like peptide-1 (GLP-1), and alters the microbiome.⁵⁶ The GLP-1 receptor is also expressed in the adipose tissue, and GLP-1 promotes adipogenesis by upregulation of adipocyte-specific markers and transcription factors.⁵⁷ This may explain why metformin did not decrease PAT volume, although it had a beneficial effect on metabolic parameters.

In our study, a T2DM patient cohort was selected to analyze the effect of insulin resistance on the prevalence of NAFLD. The control subjects did not have DM or any pancreatic, liver, or cardiovascular disease, or history of alcohol consumption, but they were matched for age, sex, BMI, and serum lipids. Therefore, NODM was the only variable in our study that could influence the prevalence of

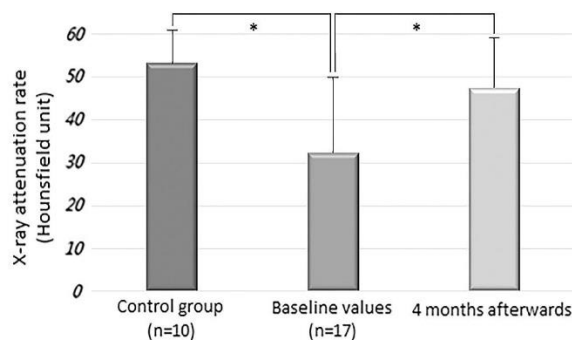


FIG. 2. The radiation absorption of the liver in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. The X-ray attenuation rate during a native CT examination was measured in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. Data expressed as mean values ± SD. * $P < 0.05$. SD, standard deviation.

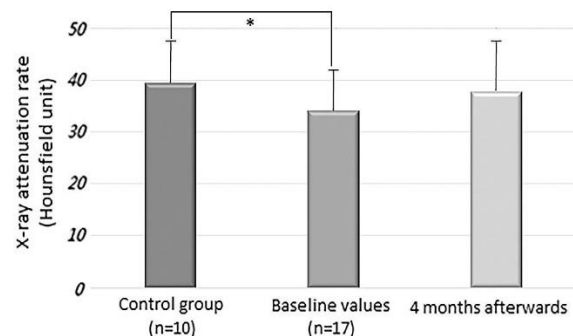


FIG. 3. The radiation absorption of the pancreas in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. The X-ray attenuation rate during a native CT examination was measured in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. Data expressed as mean values ± SD. * $P < 0.05$.

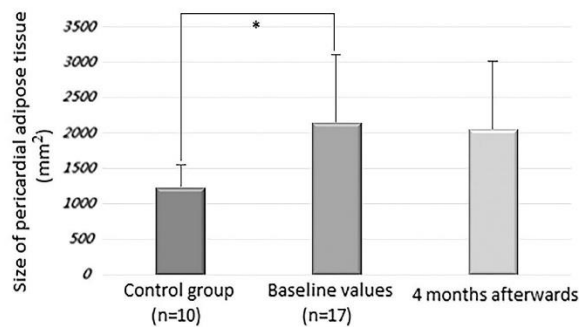


FIG. 4. The size of pericardial adipose tissue in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. A native CT examination was measured in new-onset type 2 diabetic patients before and 4 months after the start of metformin therapy and in control subjects. Data expressed as mean values \pm SD. * $P < 0.05$.

NAFLD. Patients with NODM were enrolled in this prospective study if they had been diagnosed within 1 month, they consumed no alcohol, and their medical records showed no pancreatic, liver, or cardiovascular disease, inherited disorders of fat metabolism, antidiabetic medication or pregnancy, or malignant disease. The aims of the study were (1) to analyze the effect of early phase insulin resistance on the development of NAFLD and NAFPD and (2) to investigate the effect of newly introduced metformin therapy on the degree of fat content in the liver and pancreas and on PAT size.

Sixty-nine percent and 62% of T2DM patients had NAFLD as defined by ultrasound in two previous studies,^{8,9} and 87% of the NAFLD cases were confirmed histologically in the latter study. However, these studies featured a cross-sectional design that included diabetic patients with variable disease lengths. We involved NODM patients diagnosed within 1 month before enrollment in our study. Overall, 64.7% of newly diagnosed diabetic patients in our investigation had NAFLD. This means that NAFLD is already present in the early phase of DM. The high prevalence of NAFLD in our study can be explained by the fact that our patients had several risk factors. They were generally overweight and had elevated cholesterol and triglyceride levels. However, BMI, serum cholesterol, and triglyceride levels were also above normal in the control group, and there were no significant differences between the control group and the DM group. In contrast, only 10% of the control subjects had NAFLD. Further, the radiation absorption of the liver was significantly lower, indicating a higher amount of fat in the liver in patients with T2DM compared with the control group. This increased amount of fat, therefore, can be attributed to insulin resistance after excluding other risk factors. It was demonstrated that insulin resistance is already present at least 5 years before overt diabetes in populations with a high prevalence of T2DM.⁵⁸ The high prevalence of NAFLD can also be explained by the fact that we defined fatty liver by measuring radiation absorption on CT, which is more sensitive, specific, and operator-independent compared with ultrasound.

NAFPD has been poorly investigated compared with NAFLD, although interest is increasing among researchers. Reports on the relationship between NAFPD and β -cell

function are inconsistent. Some studies indicate that pancreatic lipid content is negatively associated with insulin secretion in nondiabetic subjects²⁷ or individuals with prediabetes,²⁶ whereas others suggest that there is no relationship between β cell function and pancreatic fat in prediabetic⁵⁹ or diabetic subjects.²⁷

Pancreatic fat content can be studied with multiple diagnostic modalities. A histological examination requires a pancreatic biopsy. However, this is invasive, and there are complications associated with it. Ultrasonography is cheap and easily available, but a relatively insensitive measure of pancreatic fat content. More recently, expensive MRI techniques have been used to assess pancreatic fat deposition. A native CT scan was employed in our study to measure the amount of pancreatic steatosis using radiation absorption correlated to the spleen.

Diabetic patients have been demonstrated to have higher pancreatic fat content as measured by magnetic resonance spectroscopy^{27,31} and dual-echo magnetic resonance chemical shift imaging.⁶⁰ In contrast, Saisho et al. found that pancreatic fat content was not significantly increased in T2DM.⁶¹ Overall, 82.3% of NODM patients were diagnosed as having NAFPD based on the diagnostic criteria, whereas NAFPD was only detected in 20% of the control population in our study. Since the control group was matched for age, sex, BMI, and serum lipids with the T2DM group, NAFPD may be a consequence of insulin resistance.

Newly (<1 month) diagnosed T2DM patients were enrolled in the study to assess the effect of metformin on NAFLD and NAFPD. Metformin is the first-line agent for the treatment of diabetes and the most popular antidiabetic agent worldwide. The effects of metformin on NAFLD have been evaluated in several studies, with some of them showing a beneficial effect on aminotransferase levels or liver histological alterations.^{2,62-64} To our knowledge, no study has ever evaluated the effect of metformin on hepatic fat content measured by tissue attenuation during unenhanced CT examination. Four-month-long metformin treatment significantly reduced fat content in the liver in our study. Metformin also improved glycemic control and insulin resistance, as measured by HOMA-IR, and lowered serum cholesterol level, the results of which can partly be attributed to its beneficial effects. However, metformin therapy did not reduce pancreatic fat content and PAT size.

In conclusion, NAFLD, NAFPD, and increased PAT were detected in the majority of the NODM patients. Metformin therapy lowered the amount of fat in the liver in parallel with the improvement in metabolic parameters but did not decrease the level of adipose tissue in the pancreas nor reduce PAT size. Metformin therapy started early in the course of diabetes may be beneficial for the prevention of the late consequences of NAFLD.

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Author Disclosure Statement

The article has been read, understood, and approved by all the authors. The authors declare that this article is not under

review elsewhere and that it has not been published earlier. They have no commercial associations that might represent a conflict of interest in relation to the submitted article.

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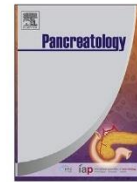
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Annex III.



Exocrine pancreatic insufficiency in type 1 and type 2 diabetes mellitus: do we need to treat it? A systematic review

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ABSTRACT

The exocrine and endocrine pancreata are very closely linked both anatomically and physiologically. Abdominal symptoms such as nausea, bloating, diarrhea, steatorrhea, and weight loss can often occur in diabetic patients. Impairments of the exocrine pancreatic function seem to be a frequent complication of diabetes mellitus; however, they are largely overlooked. The aim of this paper is to provide an overview of the current concepts of exocrine pancreatic insufficiency (PEI) in diabetes mellitus. The prevalence and symptoms of PEI in diabetes mellitus, the pathomechanism, and difficulties of diagnosis and therapy of PEI are summarized in this systematic review.

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Introduction

The exocrine and endocrine pancreata are very closely linked both anatomically and physiologically. Pathological conditions in the exocrine tissue can therefore cause an impairment of endocrine function and vice versa [1]. Pancreatic exocrine insufficiency (PEI) is defined by a deficiency of exocrine pancreatic enzymes resulting in an inability to maintain normal digestion [2]. The primary function of pancreatic enzymes is the hydrolysis of proteins (trypsinogens, proelastase, mesotrypsin), carbohydrates (α -amylase), lipids (lipase) and nucleotids (DNase, RNase). Chronic pancreatitis 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 [3]. Furthermore, PEI has been demonstrated to be present in a considerable percentage (10–74%) of patients with diabetes mellitus [4,5]. However, the significance of this findings was questioned and it is not clear, whether the presence of diabetes causes any symptoms or requires any treatment [6].

Abdominal symptoms such as nausea, bloating, diarrhea,

steatorrhea, and weight loss can often occur in diabetic patients [4]. These symptoms may be attributed to the side-effects of the metformin they are taking, the autonomic neuropathy on bowel function, small bowel bacterial overgrowth, celiac disease, or PEI. Impairments of the exocrine pancreatic function seem to be a frequent complication of diabetes mellitus; however, they are largely overlooked. Greater knowledge and awareness are required in testing and diagnosing this condition. Previous studies have raised the possibility that the replacement of pancreatic enzymes in exocrine insufficiency improves related symptoms and may aid glucose control.

The aim of this paper is to provide an overview of the current concepts of PEI in diabetes mellitus.

Search strategy

The systematic review was conducted following the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement [7]. A systematic search was performed in 3 databases, Pubmed, Embase and Cochrane 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 31st of January

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Study selection

Selection of the studies was conducted by two investigators (G.Zs. and L.C.) 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 III/C diabetes 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 judgement, then a committee of three other researchers was invited to draw a conclusion. Database searches yielded altogether 1055 articles (EMBASE: 67; PubMed: 701; Cochrane: 287). The flow-chart diagram (Fig. 1) shows the strategy and results of the study selection.

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 (Table 1a) [6,8–15]. 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. Fecal elastase-1 (FE-1) test measures fecal levels of elastase-1, a proteolytic enzyme produced by pancreatic acinar cells. Fecal level of elastase-1 correlates with the output of other pancreatic enzymes, it is highly stable in

feces and easy to measure [16]. FE-1 demonstrated good sensitivity and specificity in moderate and severe PEI [17,18]. Nowadays, therefore, 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 (Table 1b) [4–6,19–32].

The prevalence of PEI in both types of diabetes is very heterogeneous. However, most of these studies did not exclude cases with previous pancreatic disease, thus leading to a possible bias. In two recent studies, the prevalence of PEI in DM was less frequent than in previous studies, probably because pancreatic (type 3c, according to the new classification of American Diabetes Association: type 4 [33]) diabetes was excluded [28,29]. Low FE-1 was measured in only 5.4% of 150 consecutive type 1 and 2 diabetic patients after excluding patients with excessive alcohol consumption, medical history of abdominal surgery, other known reasons for malabsorption, previous pancreatic disease and DM lasting <5 years [28]. In another recent study, PEI was diagnosed with FE-1 measurement in 16.8% of type 2 diabetic patients after excluding patients with an abnormal pancreatic morphology [29]. Indeed, the prevalence of chronic pancreatic diseases among diabetic patients might be high because recent discussions have suggested that pancreatic diabetes (type 4) has been underestimated in the past and that it might cause about 8% of all diabetes cases [34].

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 [35,36]. Reduced pancreas size in patients with DM was demonstrated by abdominal ultrasonography, computed tomography or magnetic resonance imaging (MRI) [37–43]. Ductal changes are detected by endoscopic retrograde cholangiopancreatography in 76% of diabetics.

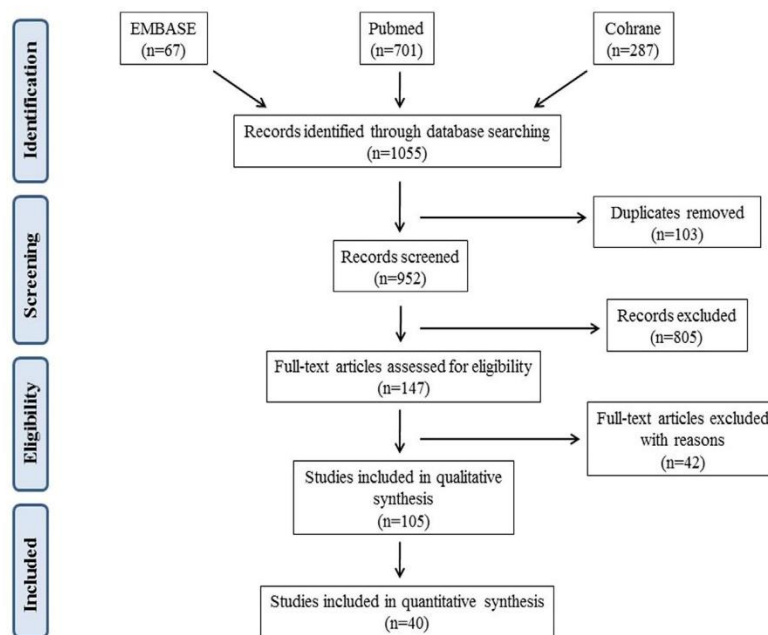


Fig. 1. The flow-chart diagram shows the strategy of the study selection.

Table 1A

Results of direct pancreatic function tests in patients with diabetes mellitus.

Author	Subjects/diabetes type	Methods	Results
Pollard et al., 1943 [8]	13	Amylase and lipase after pancreozymin-secretin stimulation	62% reduced
Chey et al., 1963 [9]	50 diabetic patients; 13 juvenile type	Amylase and lipase after pancreozymin-secretin stimulation	Low amylase output in diabetes: 36%; in juvenile diabetes: 77%
Vacca et al., 1964 [10]	55 diabetic patients (22 insulin-treated)	Diastase and bicarbonate after secretin stimulation; fecal fat	73% abnormal; correlation with age, no correlation with fecal fat
Frier et al., 1976 [11]	20 type 1, 7 type 2, 13 controls	Stimulation with iv secretin and CCK-PZ	PEI: 80% IDDM; correlation with diabetes duration
Harano et al., 1978 [12]	53 type 2, 4 type 1, 18 controls	Secretin-pancreozymin test	Diabetes: 69% deficient enzyme output; correlation with diabetes control
Lankisch et al., 1982 [13]	53 type 1	Secretin-pancreozymin test	Diabetes: 43% impaired function
Bretzke et al., 1984 [14]	60 insulin-treated type 2 diabetic patients	Secretin-pancreozymin test	Diabetes: 27% "mild PEI"
El Niewihi et al., 1988 [15]	10 type 2 diabetic patients with diarrhea and neuropathy	Secretin and CCK test	Enzyme and bicarbonate reduction in all subjects
Hahn et al., 2008 [6]	33 type 1	Secretin and CCK test	33% mild enzyme reduction

CCK-PZ: Cholecystokinin-pancreozymin; IDDM: Insulin-dependent diabetes mellitus; PEI: Exocrine pancreatic insufficiency.

Table 1B

Results of indirect pancreatic function tests in patients with diabetes mellitus.

Author	Subjects/diabetes type	Methods	Results
Hardt and Kloer 1998 [19]	128 type 1 and 2	Fecal chymotrypsin	45% < 6 U/I
		Fecal elastase 1	46% < 200 µg/g
Hardt et al., 2000 [5]	39 type 1	Fecal elastase 1	74% < 200 µg/g
	77 type 2		36% < 200 µg/g
Icks et al., 2001 [20]	112 type 1	Fecal elastase 1	54.5% < 200 µg/g
Rathmann et al., 2001 [21]	544 type 2	Fecal elastase 1	30.3% < 200 µg/g
Hardt et al., 2003 [22]	323 type 1	Fecal elastase 1	51% < 200 µg/g
	697 type 2		35% < 200 µg/g
Nunes et al., 2003 [23]	42 type 1 and 2	Fecal elastase 1	36% < 200 µg/g
Cavalot et al., 2004 [24]	66 type 1	Fecal elastase 1	26% < 200 µg/g
Yilmaztepe et al., 2005 [25]	32 type 2	Fecal elastase 1	28% < 200 µg/g
Ewald et al., 2007 [26]	546 type 2	Fecal elastase 1	21.1% < 100 µg/g
Hahn et al., 2008 [6]	33 type 1	Fecal elastase 1	33% < 200 µg/g
Larger et al., 2012 [27]	195 type 1, 472 type 2	Fecal elastase 1	23% < 200 µg/g
Vujanovic et al., 2013 [28]	50 type 1, 100 type 2	Fecal elastase 1	5.4% < 200 µg/g
Terzin et al., 2014 [29]	101 type 2	Fecal elastase 1	16.8% < 200 µg/g
Cummings et al., 2015 [4]	288 type 2	Fecal elastase 1	10% < 200 µg/g
Shivaprasad et al., 2015 [30]	89 type 1, 95 type 2	Fecal elastase 1	31% < 200 µg/g
Kangra et al., 2016 [31]	315 type 2	Fecal elastase 1	5.1% < 100 µg/g and 5.1% < 200 µg/g
Oscarsson et al., 2017 [32]	10 type 1, 38 type 2	Fecal elastase 1	33% < 200 µg/g

Interestingly, these ductal changes do not correlate with DM type, DM duration or age (Table 2) [35–48].

Pathophysiology

The mechanism of exocrine pancreatic insufficiency in diabetes is multifactorial (Fig. 2). 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 [49]. Moreover, decreased pancreatic volume and PEI were shown to correlate in patients with DM [43,50,51]. (2) Acute hyperglycemia was demonstrated to inhibit basal and cholecystokinin-stimulated pancreatic enzyme secretion with an insulin-independent mechanism [52]. (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 seC-p38 mitogen-activated protein kinase pathway, resulting in pancreatic fibrosis [53]. (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 [29]. (6) Autonomic neuropathy may give rise to impaired enteropathic reflexes and PEI [27,54,55]. Moreover, (7) viral infections [56], (8) autoimmunity [57], or (9) genetic changes, as single-base deletion in the variable number of tandem repeats containing exon 11 of the carboxyl ester lipase gene [58] could increase simultaneous damage to exocrine and endocrine tissue.

The higher prevalence of PEI in type 1 diabetes can be explained by the more severe insulin deficiency, longer disease duration, and higher rate of microvascular complications characterized by type 1 DM.

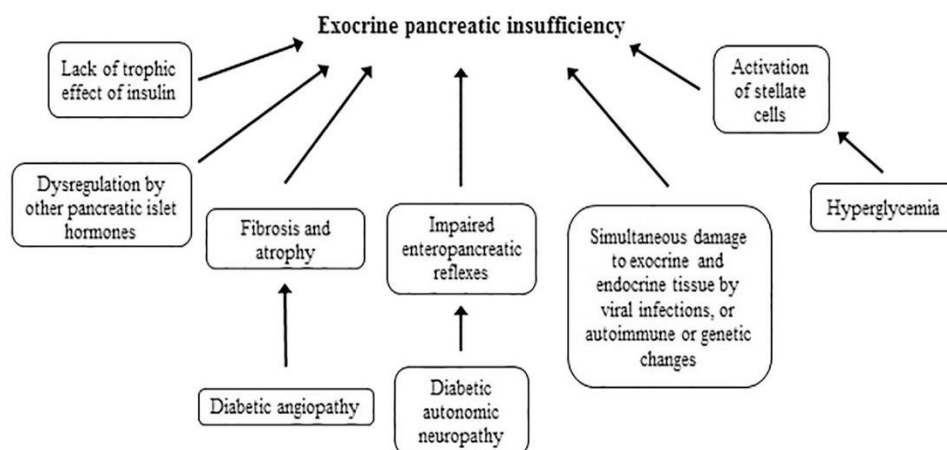
The correlation between diabetes duration and the prevalence of PEI is contradictory. Previous studies have described an association or at least a weak correlation between low FE-1 level in type 2 DM and age of onset of diabetes, relatively long diabetes duration, and relatively high glycosylated hemoglobin (HbA1c) concentration, suggesting that exocrine dysfunction is a long-term complication of diabetes [22,59]. However, studies have demonstrated that there is no relationship between fecal elastase concentration and diabetes duration [60]. Otherwise, an inverse correlation was described between diabetes duration and HbA1c levels, and a positive correlation was reported between C-peptide and FE-1 levels [59]. A long-term follow-up study suggested that a mild to

Table 2

The prevalence of morphologic changes of the exocrine pancreas in diabetes mellitus.

Author	Year	Subjects	Methods	Results
Blumenthal HT et al. [44]	1963	3821 autopsy cases	Morphology	Prevalence of pancreatitis: - In diabetics: 11.2%; - In non-diabetics: 5.3%
Putzke HP et al. [45]	1986	100 diabetic and 100 non-diabetic autopsy cases	Histopathology	Lipomatosis: - In diabetics: 75%; - In controls: 60%
Gilbeau JP et al. [37]	1992	20 type 1, 37 type 2	CT scans	Pronounced lobulation, small size compared to controls
Alzaid A et al. [39]	1993	14 type 1, 43 type 2	Ultrasound	Small size compared to controls; type1<type2<controls
Nakanishi K et al. [40]	1994	36 type 1, 43 type 2	ERCP	Changes like CP: - type 1: 40% - type 2: 9%
Klöppel G et al. [36]	1996	type 1	Histology	Fibrosis, atrophy, fatty infiltration
Foulis AK et al. [35]	1997	type 1	Histology	Fibrosis, atrophy, fatty infiltration
Altobelli E et al. [38]	1998	60 type 1	Ultrasound	Small size compared to controls; dependent on duration
Hardt PD et al. [41]	2002	38 type 1, 118 type 2	ERCP	Changes like CP: type 1 > type 2, up to 75%
Williams et al. [47]	2007	12 male patients with type 1 and 12 healthy controls	MRI	Pancreatic volume showed a 48% reduction in long-standing type 1 diabetes as compared with age-matched normal subjects.
Bilgin M et al. [42]	2009	82 type 1 and type 2	MRI/MRCP	Changes like CP
Philippe et al. [43]	2011	24 type 1 and 28 type 2	CT scans	The pancreatic volume, 42 cm (25–57 cm), was decreased in most patients
Williams et al. [48]	2012	20 male recent-onset type 1 diabetes patients and 24 male healthy controls	MRI	Pancreatic volume is reduced by 26% in type 1 diabetes
Burute N et al. [46]	2014	32 type 2 and 50 normoglycemic individuals	MRI	Patients with type 2 DM had significantly lower pancreatic volume than normoglycemic individuals ($p < 0.001$)

ERCP: endoscopic retrograde cholangiopancreatography; CP: chronic pancreatitis; CT: computed tomography; MRI: Magnetic Resonance Imaging; MRCP: Magnetic Resonance Cholangiopancreatography.

**Fig. 2.** The mechanism of exocrine pancreatic insufficiency in diabetes mellitus.

moderate exocrine pancreatic insufficiency is due to an early event in the course of DM and does not progress [61].

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 significantly decreased, however, the phosphorylation of p70S6K1, 4 E-BP1, ERK1/2, and protein ubiquitination were increased significantly compared to control group [62]. Presumably, that these factors are liable for decreased enzyme synthesis and pancreatic atrophy.

Symptoms of PEI in diabetes

The main clinical symptoms of PEI are due to the maldigestion and malabsorption of fat, including steatorrhea, abdominal pain,

flatulence, bloating and weight loss [4]. As a consequence of malnutrition, PEI is associated with low serum levels of micronutrients, lipid soluble vitamins (vitamins A, D, E, and K), trace elements, albumin, prealbumin and lipoproteins [2,29,63–74]. The low level of serum vitamin D leads to osteoporosis and an increased risk of fractures [75]. Protein-energy malnutrition and malabsorption of vitamin D and other micronutrients may result in a higher risk of infection due to their associated effects on innate and adaptive immune responses [76].

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 [77–79]. In a recent study by Cummings et al. [4], 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. Furthermore, steatorrhea was a poor marker of PEI in diabetes in this study, since only the minority of patients with steatorrhea had a low fecal elastase level. One would logically expect that diabetic patients with PEI experience weight loss, lower body weight and BMI. However, there were no significant differences in BMI between diabetic patients with a decreased or normal PE-1 concentration [4,29]. Inconsistent with these findings, the size of the pancreas did not correlate with BMI among diabetic patients in another study [37]. Furthermore, PEI detected by low FE-1 concentrations is frequent even in obese diabetic patients [23,80], and diabetic individuals with excess weight (BMI >25) may be at increased risk for PEI [25].

Diagnosis of PEI

PEI is suggested by clinical symptoms or poor glycemic control despite an adequate diet, antidiabetic therapy and patient adherence [24,29]. 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 [81–83]. FE-1 concentration correlates with the severity of PEI: a level of less than 200 µg/g stool indicates moderate PEI, while a level of less than 100 µg/g stool indicates severe PEI [84]. FE-1 is not sufficiently sensitive in mild PEI, but if FE-1 level is decreased, there is a strong chance of revealing changes in the pancreatic duct system and steatorrhea [83,85].

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 [86]. Its accuracy is similar to FE-1 in diagnosing PEI [87].

Coefficient of fat absorption (CFA) is another gold standard test for PEI [88], although it has not been evaluated in DM. During the 72-h stool collection period, the patient consumes 100 g of fat per day. Fat malabsorption is diagnosed at >7 g of fat/100 g of stool/day, with severe steatorrhea at ≥15 g/day. However, the diet is cumbersome, the 3-day stool collection is inconvenient for both patients and laboratory staff, and therefore CFA is not used in daily clinical practice. It is utilized to evaluate the effectiveness of pancreatic enzyme replacement therapy (PERT) in PEI [89].

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

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. Three small trials studied the efficacy of PERT in patients with diabetes mellitus secondary to chronic pancreatitis [90,91]. Treatment with PERT demonstrated a significant reduction in post-prandial plasma glucose and glycosylated hemoglobin at 6 months versus baseline values in patients with diabetes due to tropical calculous pancreatitis [92]. In contrast, PERT did not improve mean glucose values; it produced potentially life-threatening disturbances in glucose control among insulin-dependent diabetic patients due to chronic

pancreatitis [93]. However, a recent double-blind, randomized, placebo-controlled trial of PERT in patients with PEI due to chronic pancreatitis demonstrated that the efficacy outcomes and adverse event profile for PERT were comparable between patients with and without diabetes [94]. A larger multicenter, double-blind, randomized, placebo-controlled trial demonstrated that PERT was safe, but has no effect on glycemic control in insulin-treated diabetic patients with FE-1 <100 µg/g [26]. Reduction in mild to moderate hypoglycemic episodes was revealed after 16 weeks of treatment with four capsules of 10 000 FIP units of pancreatin with main meals and two capsules of 10 000 FIP units of pancreatin with snacks, suggesting a more stable control of insulin therapy. However, this study might be criticized. First, patients were selected according to the presence of PEI irrespective of PEI-related symptoms. Second, the applied dose of pancreatin might be low. Recent guidelines [93–96] 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 [94,97].

Nutrient-induced glucose-dependent insulinotropic polypeptide (GIP) response is diminished in patients with PEI [98]. PERT has been demonstrated to reverse an impaired GIP response and therefore to restore the incretin effect of fat [98]. 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 [99]. Furthermore, patients with diabetes mellitus have an increased risk of bone fractures [100]. 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 [26].

However, there are several limitations to this systematic review. Firstly, the prevalence of PEI in both types of diabetes is very heterogeneous, ranging between 5.1 and 80%. Secondly, studies applied the gold standard direct pancreatic function test in the measurement of PEI are limited to a small number of patients because of the invasive nature of the test. Thirdly, most of these studies did not exclude cases with previous pancreatic disease, thus leading to a possible bias. Fourth, PEI seems to be frequent in DM, data on the occurrence of the symptoms of PEI in diabetes are limited. Furthermore, only a very limited number of publications have investigated the effectiveness of PERT in PEI associated with diabetes, and the results are contradictory.

Conclusion

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.

Abbreviations

DM	Diabetes mellitus
PEI	Exocrine pancreatic insufficiency

FE-1	Fecal elastase-1
HbA1c	Glycosylated hemoglobin
FIP	International Pharmaceutical Federation
SCT	Secretin-crelulin test
PSCs	Pancreatic stellate cells
GI	Gastrointestinal
PERT	Pancreatic enzyme replacement therapy
GIP	glucose-dependent insulinotropic polypeptide
MRI	Magnetic Resonance Imaging
CFA	Coefficient of fat absorption

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