Pancreatic Elastase Levels in Feces As A Marker of Exocrine Pancreatic Function in Patients With Diabetes Mellitus

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ABSTRACT

Objective: The measurement of pancreatic elastase (PE) in feces is used widely to screen for pancreatic exocrine insufficiency. The aim of our study was to evaluate the relationship of PE with residual beta cell secretion and metabolic control in patients with diabetes mellitus.

Method: We determined the presence of PE in specimens via enzyme-linked immunosorbent assay (ELISA), whereas serum fasting glucose, C-peptide, amylase, lipase, triglycerides, total 25(OH)-vitamin D, C-reactive protein (CRP), and hemoglobin A_{1c} (HbA_{1c}) concentrations were assayed using routine laboratory tests.

Results: PE values in 48 patients with diabetes were significantly lower than in 24 healthy volunteers ($P = .001$). In one-third of participants with diabetes mellitus, PE were less than 200 μg per g, indicating pancreatic functional insufficiency. Among the patients in the cohort, PE correlated positively with C-peptide levels ($P = .04$), lipase ($P = .009$), CRP ($P = .04$), sex ($P = .03$), and BMI ($P = .02$) but not significantly with duration of diabetes ($P = .81$) or levels of HbA_{1c} ($P = .87$), amylase ($P = .06$), total 25(OH)-vitamin D ($P = .16$), or triglycerides ($P = .52$).

Conclusion: Our results demonstrated a strong association of diabetes with low PE levels.

Keywords: pancreatic elastase, pancreatic exocrine insufficiency, diabetes mellitus, feces, ELISA, biomarker

Despite the large functional reserve of the exocrine pancreas, which was estimated to be 3 times larger than the functional reserve of the endocrine pancreas, we discovered an insufficient level of pancreatic exocrine function in a significant percentage of patients with diabetes mellitus. The molecular basis for this phenomenon is not completely clarified, particularly regarding the type of cell that is the source of the disease and the basic pathological process. Several pathophysiological concepts and hypotheses have been suggested[1-7] in various attempts to explain the dysfunction of the exocrine pancreatic function in patients with diabetes.

The aim of this study was to determine the frequency and level of exocrine pancreatic function insufficiency in the patients with type 1 and type 2 diabetes mellitus (T1DM and T2DM, respectively), by determining the concentration of pancreatic elastase (PE) in the feces. As an indirect assay for evaluation of pancreatic exocrine function, PE testing has become a standard diagnostic tool with good correlation to the results of direct functional tests and morphological changes in the pancreas discovered via imaging techniques.[8,9] The main disadvantage of determining concentration of PE is low sensitivity in mild to moderate insufficiency.[10] Another limitation of the clinical usefulness of PE is posed by the difficulty in differentiating pancreatic from nonpancreatic malapsorption.[11] In assessing the level of insufficiency of exocrine pancreatic function, we applied the

Abbreviations

T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; PE, pancreatic elastase; Hba_{1c}, hemoglobin A_{1c}; CRP, C-reactive protein; $EDTA\text{-}Na_2$, ethylenediaminetetraacetic acid disodium; ELISA, enzyme-linked immunosorbent assay; CVs, coefficients of variation; BMI, body mass index; ERCP, endoscopic retrograde cholangiopancreatogram; GGT, gamma-glutamyltransferase; NA, not applicable; IQR, interquartile range; B (95% CI), unstandardized coefficient with 95% confidence interval; β, standardized coefficient

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standard cut-off value, determined based on a large group of patients with exocrine pancreatic insufficiency of different etiologies. According to the applied cut-off value, values between 100 μg per g and 200 μg per g of feces indicate mild to moderate insufficiency of exocrine pancreatic function, and values between 100 μg per g and 20 μg per g of feces are associated with severely reduced function, whereas values below 20 μg per g of feces are referred to as indicating extremely reduced exocrine pancreatic function.

To assess the significance of PE levels in the studied group with diabetes, we examined the connection between PE levels and glucose metabolism based on fasting serum glucose and hemoglobin A_{1c} (HbA_{1c}) levels as indicators of metabolic control. Also, to assess the trophic role of insulin in pancreatic exocrine cells, we determined the connection between PE levels in the feces and the concentration of C-peptide in the serum as a measure of the residual function of pancreatic β-cells. We examined the secretory function of the exocrine pancreas in patients with diabetes by recording levels of amylase and lipase, as well as by investigating the possible connection of these enzymes with PE levels in feces. We measured C-reactive protein (CRP) as an indicator of the acute inflammation phase, to evaluate the inflammatory status of the pancreas in an attempt to detect the stage of deterioration of endocrine and exocrine pancreatic dysfunction. By determining the values of triglycerides, we evaluated the involvement of altered lipid metabolism in the pathogenesis of exocrine pancreatic function insufficiency.

We examined the prevalence of insufficient concentrations of total 25(OH)-vitamin D in patients with diabetes and the possible connection between low levels of PE in the feces and insufficient levels of total 25(OH)-vitamin D, as a measure of qualitative malnutrition, with respect to lipids. Further, we estimated the interconnection and prognostic significance of analyzed biochemical parameters on the PE levels in feces. Also, we evaluated a possible correlation between levels of PE with the age and sex of patients, duration of diabetes, and diabetes type in the group of patients with diabetes.

Materials and Methods

In this article, we cover a population of patients with diabetes who underwent a routine checkup to the Clinic for Endocrinology, Diabetes and Metabolic Disorders, Clinical Center of Serbia in the period from June 2009 through October 2009. The study population comprises a cohort of 48 patients (30 women and 18 men) aged 22 years to 82 years. In the group of patients with diabetes, there were 10 patients with T1DM and 38 with T2DM. The duration of illness ranged from 1 year to 36 years, with a median of 12 years. During this study, 33 patients were taking insulin therapy, 12 patients used oral antidiabetic drugs, and 3 patients were taking combination drug therapy. Our study also included a control group that consisted of 24 healthy volunteers (20 women and 4 men) aged 25 years to 76 years. This group consisted of persons without diabetes or any presence of chronic or acute diseases of the gastrointestinal tract. We collected specimens of blood and feces from the control group from July 2009 through October 2009.

Blood specimens were collected between 7 AM and 10 AM, after a period of fasting. After venipuncture and the time required for coagulation, we centrifuged the blood specimens for 15 minutes at 1500 *g* at room temperature. Serum aliquots were separated and immediately frozen at -70 °C until determination of C-peptide, glucose, lipase, amylase, total 25(OH)-vitamin D, triglycerides, and CRP. HbA1c was measured on the same day from whole blood specimens with ethylenediaminetetraacetic acid disodium ($EDTA\text{-}Na_2$) as an anticoagulant. After the stool specimens arrived at the laboratory, they were stored at -20 °C until the extraction and determination of PE. According to the manufacturer instruction, the specimen size was greater than the minimum required and the stool specimens were well formed (ie, they were not of watery consistency). We performed PE extractions, following instructions provided by the kit manufacturer. Extracts of stool specimens were tested in duplicate; results were reported as the mean of the 2 measurements. Procedures of extraction and determination of PE were repeated in all specimens with borderline or low PE results.

PE levels were determined using a "sandwich" enzyme-linked immunosorbent assay (ELISA) kit, which combines the use of 2 monoclonal antibodies that are specific to human PE (ScheBoBiotech AG). Biochemical parameters were analyzed by using commercial tests because they are available on 2 analyzers: the integrated biochemical and immunochemical analyzer *ci*8200 Architect (Abbott Laboratories Inc) and the multichannel immunochemical analyzer Cobas e601 (F. Hoffman-La Roche Ltd). We regularly checked the reliability of the measurement results

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Table 1. Baseline Demographic and Clinical Data Distribution of Patients With Diabetes and Control Individuals and the Skewness of Data for Determined Parameters in the Studied Groups

Variable	Patients With Diabetes (n = 48)		Control Individuals (n = 24)	
	Median (IQR)	*P* Value[a]	Median (IQR)	*P* Value[a]
Age (y)	61 (20)	.02	50 (21)	.84
Sex				
Male	18 (37.5)		4 (16.7)	
Female	30 (62.5)		20 (83.3)	
Type of diabetes				
Type 1	10 (21)		NA	
Type 2	38 (79)		NA	
Duration of diabetes (y)	12 (15)	.02	NA	NA
Body mass index (kg/m^2)	26 (6)	<.001	24 (6)	.53
Pancreatic elastase (μg/g)	386 (498)	<.001	648 (481)	.04
C-peptide (pmol/L)	311 (542)	<.001	460 (223)	<.001
Glucose (mmol/L)	8.7 (4.5)	.07	4.9 (0.6)	.06
HbA_{1c} (%)	9.2 (2.7)	.49	5.1 (0.7)	.50
Amylase (U/L)	46 (30)	.04	59 (34)	.03
Lipase (U/L)	22 (26)	<.001	22 (15)	.02
Triglycerides (mmol/L)	1.58 (1.13)	<.001	1.14 (0.64)	.10
C-reactive protein (mg/L)	1.82 (5.81)	<.001	0.71 (1.91)	<.001
Total 25(OH)-vitamin D (μg/L)	17.2 (14.6)	.14	23.8 (16.4)	.13

NA, not applicable; IQR, interquartile range; HbA_{1c}, hemoglobin A_{1c}.
[a]P > .05 indicates normal distribution (Shapiro-Wilk W test).

through assessment of appropriate controls and application of the internal quality control principle.

Statistical computer programs SPSS, version 11.5 for Windows (SPSS, Inc) and MedCalc, version 9.5.2.0 (MedCalc Software bvba) were used for the statistical analysis and to produce the resulting data charts. $P < .05$ was determined to show statistical significance.

Results

For within-run imprecision evaluation, 3 feces samples were determined to have low (130 μg/g of feces), borderline low (225 μg/g of feces), and normal (479 μg/g of feces) PE values, for 15 replicates per run. The coefficients of variation (CVs) that we obtained were 7.5%, 6.9%, and 4.8%, respectively. We calculated the between-run imprecision from measuring PE in 3 homogenates of specimens with values of 96 ± 14.1, 203 ± 17.3, and 351 ± 27.4 μg/g of feces for 10 consecutive days. The resulting CVs were 14.6%, 8.5%, and 7.8%, respectively. Method inaccuracy was evaluated using the commercial control material with assigned value of 200 μg PE per g of feces. Concentration of PE was determined in 15 replicates, and the obtained mean and the assigned value were not significantly different according to the results of the Student's *t*-test ($P = .18$). The linearity of the PE ELISA assay was determined with the concentration of the lowest and highest standard, which were 15 μg and 500 μg PE per g of feces, respectively.

Table 1 includes the baseline demographic and clinical characteristics of the study participants divided into a group with diabetes and a group of healthy volunteers (control group). For comparison of data collected for sex, age, and body mass index (BMI) between the control and diabetes groups, we used the nonparametric Pearson chi-square test. In the control group, there were more women than in the group with diabetes, but that difference was not statistically significant ($P = .07$). The studied groups also did not statistically differ by age ($P = .08$) or by BMI ($P = .11$).

For normal distribution, we used the Shapiro-Wilk *W* test. The skewness of data for determined parameters in the diabetes and control groups is represented in **Table 1**. For

comparison of continuous variables between the 2 studied groups, we used the Student's *t*-test if the distribution was normal and the nonparametric Mann-Whitney *U*-test test for skewed continuous variables. Compared with the control group, patients with diabetes had significantly lower values (Mann-Whitney U-test, $P < .05$) of PE ($P = .001$), C-peptide ($P = .03$), and amylase ($P = .02$) but significantly higher levels of CRP ($P = .004$) and triglyceride ($P = .001$), whereas lipase values did not differ significantly ($P = .81$). Values of total 25(OH)-vitamin D in the diabetes group were significantly lower among patients with diabetes (Student's *t*-test, $P < .05$) than in the control group ($P = .001$), whereas levels of glucose and HbA1c were significantly higher ($P < .001$). The distribution of determined values of all the examined parameters in the 2 groups is shown graphically in **Figure 1**.

The existence and degree of correlation between the 2 parameters in the group of patients with diabetes was tested by using the Spearman nonparametric correlation analysis. The aim was to investigate whether there is a correlation between the observed variables and if so, to what significance of correlation is the agreement. We set the Spearman correlation coefficient to establish links between PE levels and other biochemical parameters, as well as certain patient characteristics in the group with diabetes. The results of correlations between concentrations of PE and the studied parameters are summarized in **Table 2**. The results showed that the values of PE for patients with diabetes were significantly positively correlated with levels of C-peptide, lipase, and CRP; BMI; and sex. However, the obtained correlation coefficients are between 0.25 and 0.50, indicating a low degree of correlation.

By applying multiple regression analysis with all variables entered into the model, we showed that combined levels of amylase and CRP, along with age and BMI, significantly predict the concentration of PE among patients with diabetes. We selected the stepwise regression analysis method to use in analyzing our data. The variables were checked after introduction into the model and possibly turned off if they became statistically insignificant. The results of multiple regression analysis are shown in **Table 3**.

Discussion

Reduced exocrine pancreatic function in patients with diabetes was observed in several studies,[12-15] some of which were conducted more than 20 years ago. These studies used direct measurement of pancreatic enzymes in duodenal aspirate or measurement of the concentration of immunoreactive trypsin, lipase activity, amylase, and pancreatic isoamylase activity in the serum and measurement chymotrypsin in the feces. High prevalence of exocrine pancreatic insufficiency in patients with diabetes was confirmed in many studies based on the determination of PE in the feces.[16-26] PE values below 100 μg per g were found in 11% to 30% of patients with T1DM and in 3% to 20% of patients with T2DM.

In the current study, we obtained significantly lower PE values in the group with diabetes than in the control group. A PE level of less than 100 μg per g was found in 14% of patients with diabetes, whereas values of less than 200 μg per g were obtained in 33% of patients with diabetes and in 4% of healthy volunteers. Values below 200 μg per g were obtained in 3 of 10 patients with T1DM and in 13 of 38 patients with T2DM. Some believe that diabetes secondary to illness(es) of the pancreas (diabetes type 3c) is often mistakenly classified at diagnosis as being some other type of diabetes.

A reclassification study of 1868 patients with diabetes[27] found that a high percentage of patients met diagnostic criteria for the classification in a group with type 3c diabetes (9.2%). Previous assessments claimed that type 3c diabetes accounts for only 0.5% to 1.15% of all cases of diabetes. These estimates date back to the 1960s and 1980s and probably deviate from the correct value because evaluation of the exocrine pancreas at that time was limited to highly invasive procedures (such as direct functional tests and endoscopic retrograde cholangiopancreatogram [ERCP]). If the 9.2% reclassification finding is confirmed by prospective studies, it will be likely to significantly change the current view of the epidemiology of diabetes. Consequently, based on these results, the evaluation of exocrine pancreatic function and morphologic characteristics should be included in the clinical workup of with patients with diabetes at least during the manifestation of the disease because pancreatic diabetes differs significantly in management challenges compared with T1DM and T2DM. Severe type 3c diabetes is commonly referred to as *brittle diabetes* due to a combination of persistent mild hyperglycemia and frequent episodes of hypoglycemia associated with increased peripheral and decreased hepatic sensitivity to insulin.[28]

In our research, we obtained a significantly higher percentage of low values of PE in men (55%) than in women (20%), in agreement with the earlier results, which found that

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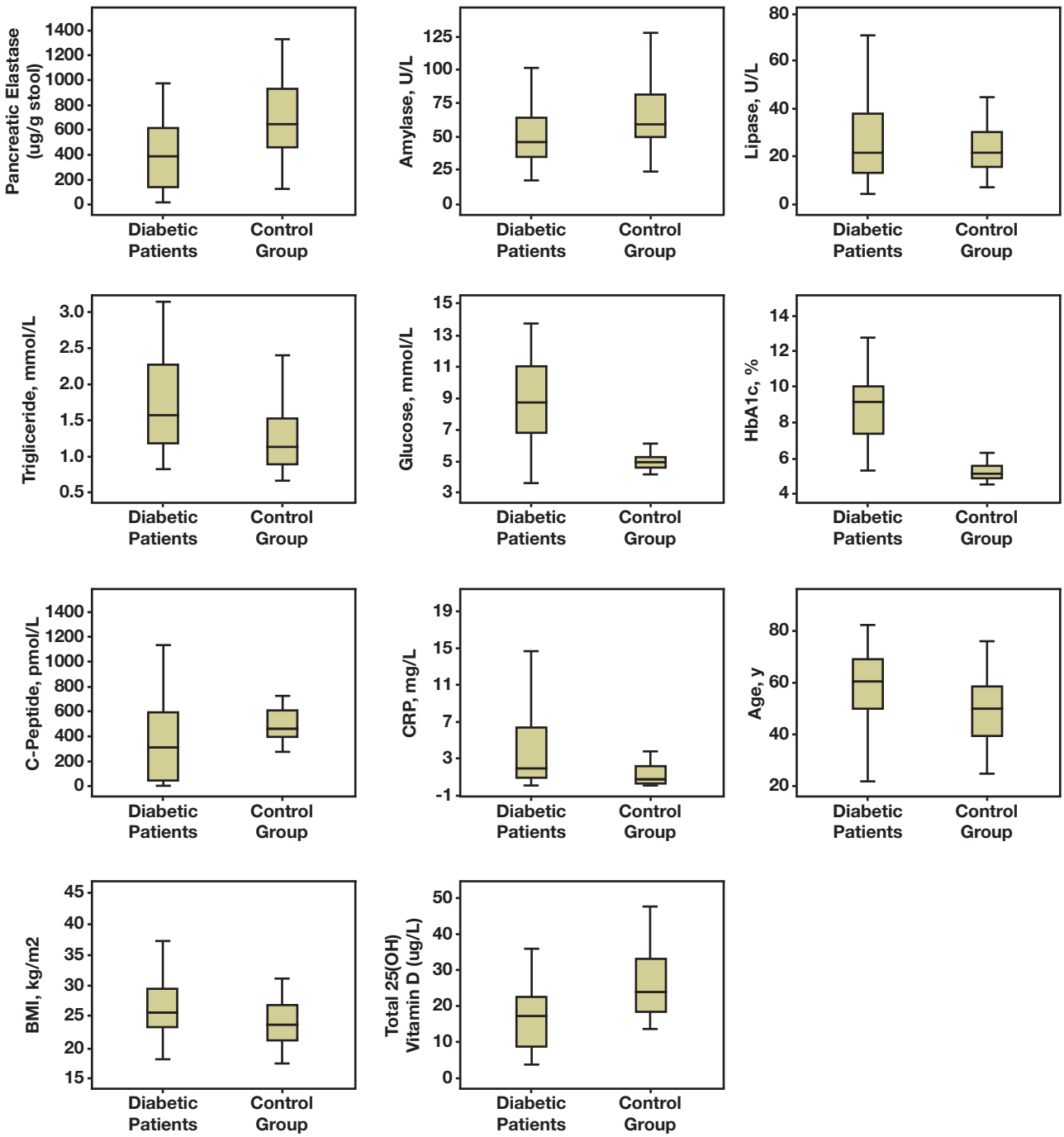

Figure 1

Distribution of values of all the examined parameters in the patients with diabetes and control individuals. Data are presented as box-and-whisker plots showing 25th, median (50th), and 75th percentile of the distribution. *HbA_{1c} indicates hemoglobin A_{1c}; CRP, C-reactive protein; BMI, body mass index*.

Table 2. Correlation Between the Concentration of Pancreatic Elastase and Other Studied Parameters in the Group of Patients With Diabetes

Parameter	Pancreatic Elastase (μg/g of Stool)	
	ρ Value[a]	*P* Value[b]
Sex	0.311	**.03**
Diabetes type	0.009	.95
Age (y)	−0.134	.36
Diabetes duration (y)	0.037	.81
BMI (kg/m^2)	0.337	**.02**
C-peptide (pmol/L)	0.297	**.04**
Glucose (mmol/L)	−0.122	.41
HbA_{1c} (%)	−0.025	.87
Amylase (U/L)	0.273	.06
Lipase (U/L)	0.373	**.009**
Triglycerides (mmol/L)	0.094	.52
C-reactive protein (mg/L)	0.302	**.04**
Total 25(OH)-vitamin D (μg/L)	0.207	.16

[a]*Indicates Spearman correlation coefficients; BMI, body mass index; HbA_{1c}, hemoglobin A_{1c}.*
[b]*Bolded values of* $P < .05$ *indicate a significant correlation between the parameters.*

the risk of having PE value of less than 100 μg per g is 3 to 4 times higher[18] and 2 times higher[19] in men than in women. This finding is often connected to increased alcohol consumption among male subject individuals, which may lead to higher incidence of subclinical chronic pancreatitis.[29]

In this study, we found no relationship between low PE values and duration of diabetes. Data in the literature are discordant regarding this relationship. Our results agree with those of some studies,[19,21,23-25] but other study results associate duration of diabetes with PE values of less than 200 μg per g.[18,20,22] In some studies, duration of diabetes was associated with exocrine failure in patients with T1DM but not in those with T2DM, linking it with the ethiology of the endocrine failure.[16] In our study, the cohort group was comprised of patients with T1DM and those with T2DM, with an almost 4-fold greater prevalence of participants with T2DM. This could be the reason why the influence of the duration of diabetes was not demonstrated. The influence of diabetes duration on the level of PE is often associated with a progressive reduction of β-cell function with time and consequent disorder of blood-glucose control. However, the results of monitoring studies of pancreatic exocrine function in patients with T1DM have shown that exocrine pancreatic insufficiency is not a clinically significant late complication of T1DM because the results of pancreosimin-secretin testing of more than 80% of patients with initially normal pancreatic function remained normal after a follow-up period of 11 years.[30] In the same study, it was observed that the disturbance of pancreatic exocrine function was generally mild. In most patients, the reduction of secretion of lipase was higher than 10% of the normal level, so usually, steatorrhea did not occur, and enzyme replacement therapy was not required. The authors concluded that mild to moderate exocrine pancreas failure may occur at an early stage of autoimmune diabetes and does not progress.

In this study, we found that the BMI value for patients with diabetes positively correlated with PE values. A greater percentage of patients with BMI of less than 25 kg per m^2 had a low PE value, which is consistent with the results of some studies.[20,25,26] This finding may be explained by exocrine insufficiency leading to malassimilation. Another study[19] used cut-off values of BMI less than 27 kg per m^2 to show that patients with diabetes and low body weight have 2-fold higher likelihood of having PE values below 100 μg per g. However, some studies did not find a significant connection.[22-24] Moreover, in other studies,[21,31] a negative correlation was found (ie, lower values of PE were found) in persons with diabetes who also have obesity. Because most patients with T2DM have obesity, this finding does not agree with a general belief that failure of exocrine pancreatic functioning leads to a loss of body weight. These researchers concluded that the low values of PE in the group with diabetes and obesity were obtained due to the low specificity of the test. However, there is a possibility that obesity and diabetes have a significant synergistic effect on the development of exocrine pancreatic function insufficiency.

Several similar studies have assessed metabolic control of diabetes expressed by HbA_{1c}, with various outcomes. For example, in a study of patients with T2DM, poor glycemic control ($HbA_{1c} \geq 7.0\%$) was associated with a higher probability of obtaining low PE values.[19] The assumption is that the lack of digestive enzymes leads to poor digestion and intraluminary malabsorption, followed by poor control of blood-glucose levels. We found no relationship between HbA_{1c} values and PE levels, in agreement with the findings of certain studies.[23-25] However, other studies[22,26] showed an inverse correlation between these 2 variables.

In this study we found a significant positive correlation between PE levels in feces and the concentration of C-peptide in serum as a marker of residual β-cell secretion, which is consistent with the results of certain studies.[22,26] In this study, 42% of patients with C-peptide values below the reference method values (<260 pmol/L) had also low levels

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Table 3. Results of Multiple Regression Analysis

	PE Value, Dependent Variable (μg/g of stool)		
Predictor Variable	*β*	**B (95% CI)**	***P* Value[a]**
Amylase (U/L)	0.45	6.69 (3.13 – 10.25)	<.001
C-reactive protein (mg/L)	0.32	9.73 (1.16 – 18.3)	.03
Age (y)	−0.32	−6.82 (-12.02 – -1.62)	.01
BMI (kg/m^2)	0.38	21.25 (5.54 – 36.72)	.009
Intercept (constant)	NA	−160.58 (-644.62 – 323.490)	.51

PE, pancreatic elastase; B (95% CI), unstandardized coefficient with 95% confidence interval; β, standardized coefficient; BMI, body mass index; NA, not applicable.
[a] *P <.05 indicates a significant predictor. R^2-adjusted: 0.39; F: 8.49; P <.001.*

of PE. One study[22] concluded that in patients with C-peptide less than 160 pmol per L, levels of PE were approximately 50% lower compared with patients with diabetes who have C-peptide values higher than 160 pmol per L (211 μg/g vs 424 μg/g), whereas the PE values in patients with diabetes and C-peptide values higher than 160 pmol per L were similar to those obtained in the control group. The same study suggests that a C-peptide value of higher than 160 pmol per L can be considered a cut-off point, above which endocrine pancreatic function does not damage exocrine function in T1DM. However, normal PE values in some patients with C-peptide values lower than 160 pmol per L indicate that insulin secretion is not the only factor that determines PE level in patients with T1DM.

In our study we found that patients with diabetes have a significant association between fecal PE levels and serum lipase activity, but amylase activity was not significantly correlated with PE. The amylase activity was significantly lower in the patients with diabetes than in the control group (51 U/L vs 65 U/L); this finding is consistent with those of other studies.[32,33] One study[34] found reduced serum levels of lipase in patients with T1DM only. The disruption in amylase and lipase activity in patients with diabetes suggests that the determination of pancreatic enzymes in this group of patients could be a useful marker in assessing disease progression.

Researchers agree that increased levels of CRP are common in patients with T2DM, gestational diabetes, and metabolic syndrome, and that there is a significant correlation between CRP and the development of T1DM and T2DM. However, in this study we found a significant positive correlation between low PE values in stool specimens and low serum CRP levels in the group of patients with diabetes (**Tables 2** and **3**).

Among the 14 patients with PE values below 200 mg per g in their stool specimens, we discovered that 11 have CRP values lower than 5 mg per L. Another study[35] found increased levels of inflammatory markers in 7 of 28 patients with chronic pancreatitis; also, higher values were found in patients who were at the disease relapse stage. That study concluded that CRP in chronic pancreatitis reflects the inflammatory status of the gland. In our study we found that as many as 6 of 7 patients with PE values lower than 100 μg per g of stool specimen have CRP values lower than 1 mg per L. We concluded that low levels of CRP in patients with low PE values are associated with advanced stages of insufficient exocrine pancreatic function when the process of fibrosis of the pancreas has advanced and is not associated with acute tissue inflammation. However, this conclusion requires confirmation in further trials.

The results of many cellular and animal studies and some human studies have linked 25(OH)-vitamin D deficiency with the pathophysiology of diabetes, suggesting that 25(OH)-vitamin D replacement therapy might be beneficial in this context.[36,37] We found significantly lower levels of total 25(OH)-vitamin D in the experimental group of patients with diabetes, compared with the control group (mean, 16.7 vs 26.1 mg/L); this finding is in agreement with data from the literature.[38,39] In our study we found that having a level of total 25(OH)-vitamin D lower than 20 mg per mL is more frequent among individuals with T2DM than in those with T1DM (68% vs 50%, respectively). Di Cesar et al[39] found a higher prevalence of 25(OH)-vitamin D deficiency in patients with T2DM (63.5%), compared with a group of patients with T1DM (36%). In the present study, there was no significant association between BMI and levels of 25(OH)-vitamin D, despite that many studies[40-42] have found that in obese patients, 25(OH)-vitamin D accumulates in adipose tissue,

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leading to low levels of 25(OH)-vitamin D in the circulation. A higher percentage of patients with T2DM are obese, compared with patients with T1DM, which may partly explain the higher prevalence of 25(OH)-vitamin D deficiency in patients with T2DM. Most patients with diabetes take multivitamin supplements that contain 25(OH)-vitamin D. Di Cesar et al[39] found that as many as 72% of patients with 25(OH)-vitamin D deficiency take multivitamins as a form of 25(OH)-vitamin D supplementation. This paradox may be explained by the lack of pancreatic exocrine function found in a large percentage of patients with diabetes. Patients with exocrine pancreatic insufficiency absorb only approximately 40% of the 25(OH)-vitamin D in the intestinal tract, whereas in healthy subjects, this rate is 80% to 90%.

In patients with diabetes, exocrine pancreatic function insufficiency can cause a characteristic clinical picture (diarrhea with steatorrhea and weight loss) and metabolic instability that require the constant modification of insulin therapy. In these cases, due to the risk of malnutrition and compromised quality of life due to gastrointestinal discomfort, it is important to start with treatment of exocrine pancreatic insufficiency, including lipid-soluble 25(OH)-vitamin D deficiency. In clinical practice, little attention is given to the fact that insufficient exocrine pancreatic function has been found in 10% to 40% of patients with diabetes, compared with approximately 4% of individuals without diabetes. The clinical relevance of exocrine pancreatic insufficiency expressed by the PE deficiency in patients with diabetes has been questioned based on a claim that it is a mild to moderate condition that does not progress.[10,30] The lack of visible clinical symptoms of exocrine pancreatic insufficiency is used as an argument for denying its importance in diabetes. Also, patients with increased excretion of lipids, manifested as increased presence of fats in the stool, usually do not spontaneously report abdominal symptoms. However, when a direct question on this subject is included in a clinical interview or a questionnaire, some patients report symptoms such as soft stools, bloating, and abdominal pain. Most of these patients showed improvement in abdominal symptoms after the start of replacement therapy with pancreatic enzymes.

The present study has some limitations that should be acknowledged and discussed. First, the study population was heterogeneous with respect to age, sex, type of diabetes, and duration of diabetes. Underlying diseases other than diabetes, such as chronic pancreatitis, cystic fibrosis, cholelithiasis (gallstones), and pancreatic cancer, could have contributed to the abnormal PE findings. Although in this study we did not collect information about diseases other than diabetes, some conclusions might be inferred on the basis of the available laboratory results. Only 4 patients with diabetes had creatinine levels higher than the normal value of 110 μmol per L, which suggests that most of the patients included in this study did not have advanced kidney disease. Further, only 4 patients had gamma-glutamyltransferase (GGT) values that were higher than normal (35 U/L for women and 55 U/L for men), which suggests that most likely, the majority of patients were not heavy alcohol users and did not have alcoholic cirrhosis or posthepatic biliary obstruction. Lack of information on multivitamin supplements taken by our cohort individuals is another limitation of this study, which could affect the accuracy of our vitamin-D-status estimation. Finally, given the small sample size of the study population, possible insufficient statistical power in the present study should be taken into consideration during interpretation of results.

The main result of the present study is that, according to our measurements of the levels of PE in feces, exocrine pancreatic function is often decreased in patients with diabetes. We found that C-peptide, lipase, and CRP levels, as well as sex and BMI, correlated with PE among the parameters that we analyzed. Although insufficient levels of exocrine pancreatic function are common in patients with diabetes, the clinical significance of this dysfunction remains a controversy. Therefore, we support suggestions that analysis of exocrine pancreatic function can be beneficial for patients with diabetes if they also experience sudden deterioration in control of glucose metabolism, weight loss, or symptoms of gastrointestinal disorder of unknown origin. **LM**

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References

1. Barreto SG, Carati CJ, Toouli J, Saccone GTP. The islet-acinar axis of the pancreas: more than just insulin. *Am J Physiol Gastrointest Liver Physiol* 2010;299:G10–G22.
2. Czakó L, Hegyi P, Rakonczay Z, Wittmann T, Otsuki M. Interactions between the endocrine and exocrine pancreas and their clinical relevance. *Pancreatology* 2009;9:351–9.

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3. Chen N, Unnikrishnan IR, Anjana RM, Mohan V, Pitchumoni CS. The complex exocrine-endocrine relationship and secondary diabetes in exocrine pancreatic disorders. *J Clin Gastroenterol* 2011;45(10):850–61.
4. Hardt PD, Ewald N. Exocrine pancreatic insufficiency in diabetes mellitus: a complication of diabetic neuropathy or a different type of diabetes? *Exp Diabetes Res* 2011;2011:761950
5. Bilgin M, Balci NC, Momtahen AJ, Bilgin Y, Klör H-U, Rau WS. MRI and MRCP findings of pancreas in patients with diabetes mellitus: compared analysis with pancreatic exocrine function determined by fecal elastase 1. *J Clin Gastroenterol* 2009;43(2):165–70.
6. Philippe MF, Benabadji S, Barbot-Trystram L, Vadrot D, Boitard C, Larger E. Pancreatic volume and endocrine and exocrine functions in patients with diabetes. *Pancreas* 2011;40(3):359–63.
7. Taniguchi T, Okazaki K, Okamoto M, et al. High prevalence of autoantibodies against carbonic anhydrase II and lactoferin in type I diabetes: concept of autoimune exocrinopathy and endocrinopathy of the pancreas. *Pancreas* 2003;27(1):26–30.
8. Stein J, Jung M, Sziegoleit A, Zeuzem S, Caspary WF, Lembcke B. Immunoreactive elastase I: clinical evaluation of a new noninvasive test of pancreatic function. *Clin Chem* 1996;42(2):222–6.
9. Löser C, Möllgaard A, Fölsch UR. Faecal elastase 1: a novel, highly sensitive, and specific tubeless pancreatic function test. *Gut* 1996;39:580–6.
10. Lankisch PG, Schmidt I, König H, Lehnick D, Knollmann R, Löhr M, et al. Faecal elastase 1: not helpful in diagnosing chronic pancreatitis associated with mild to moderate exocrine pancreatic insufficiency. *Gut* 1998;42:551–4.
11. Tod J, Fine D. Fecal elastase: a useful test for pancreatic insufficiency? *Dig Dis Sci* 2010;55:2709–11.
12. Lankisch PG, Manthey G, Otto J, Koop H, Talaulicar M, Willms B. Exocrine pancreatic function in insulin-dependent diabetes mellitus. *Digestion* 1982;25:211–6.
13. Dandona P, Freedman DB, Foo Y, Perkins J, Katrak A, Mikhailidis DP, et al. Exocrine pancreatic function in diabetes mellitus. *J Clin Pathol* 1984;37:302–6.
14. Semakula C, Vandewalle CL, Van Schravendijk CF, Sodoyez JC, Schuit FC, Foriers A, et al. Abnormal circulating pancreatic enzyme activities in more than twenty-five percent of recent-onset insulin-dependent diabetic patients: association of hyperlipasemia with high-titer islet cell antibodies. *Pancreas* 1996;12(4):321–33.
15. Kim KH, Lee HS, Kim CD, Chun HJ, Song CW, Um SH, Ryu HS, et al. Evaluation of pancreatic exocrine function using pure pancreatic juice in non-insulin-dependent diabetes mellitus. *J Clin Gastroenterol* 2000;31(1):51–4.
16. Larger E, Philippe MF, Barbot-Trystram L, Radu A, Rotariu M, Nobecourt E, et al. Pancreatic exocrine function in patients with diabetes. *Diabet Med* 2012;29(8):1047–54.
17. Hardt PD, Krauss A, Bretz L, Porsch-Ozcürümez M, Schnell-Kretschmer H, Mäser E, et al. Pancreatic exocrine function in patients with type 1 and type 2 diabetes mellitus. *Acta Diabetol* 2000;37:105–10.
18. Icks A, Haastert B, Giani G, Rathmann W. Low fecal elastase-1 in type I diabetes mellitus. *Z Gastroenterol* 2001;39:823–30.
19. Rathmann W, Haastert B, Icks A, Giani G, Hennings S, Mitchel J, et al. Low faecal elastase 1 concentrations in type 2 diabetes mellitus. *Scand J Gastroenterol* 2001;36:1056–61.
20. Hardt PD, Hauenschild A, Nalop J, Marzeion AM, Jaeger C, Teichmann J, et al. High prevalence of exocrine pancreatic insufficiency in diabetes mellitus. A multicenter study screening fecal elastase 1 concentrations in 1,021 diabetic patients. *Pancreatology* 2003;3:395–402.
21. Nunes AC, Pontes JM, Rosa A, Gomes L, Calvarheiro M, Freitas D. Screening for pancreatic exocrine insufficiency in patients with diabetes mellitus. *Am J Gastroenterol* 2003;98:2672–5.
22. Cavalot F, Bonomo K, Perna P, Bacillo E, Salacone P, Gallo M, et al. Pancreatic elastase-1 in stools, a marker of exocrine pancreas function, correlates with both residual beta-cell secretion and metabolic control in type 1 diabetic subjects. *Diabetes Care* 2004;27(8):2052–4.
23. Yilmaztepe A, Ulukaya E, Ersoy C, Yilmaz M, Tokullugil HA. Investigation of fecal pancreatic elastase-1 levels in type 2 diabetic patients. *Turk J Gastroenterol* 2005;16:75–80.
24. Laass MW, Henker J, Thamm K, Neumeister V, Kuhlisch E. Exocrine pancreatic insufficiency and its consequences on physical development and metabolism in children and adolescents with type 1 diabetes mellitus. *Eur J Pediatr* 2004;163:681–2.
25. Hahn JU, Kerner W, Maisonneuve P, Lowenfels AB, Lankisch PG. Low fecal elastase 1 levels do not indicate exocrine pancreatic insufficiency in type-1 diabetes mellitus. *Pancreas* 2008; 36(3):274–8.
26. Ewald N, Raspe A, Kaufmann C, Bretzel RG, Kloer HU, Hardt PD. Determinations of exocrine pancreatic function as measured by fecal elastase-1 concentrations (FEC) in patients with diabetes mellitus. *Eur J Med Res* 2009;14:1–6.
27. Ewald N, Kaufmann C, Raspe A, Kloer HU, Bretzel RG, Hardt PD. Prevalence of diabetes mellitus secondary to pancreatic diseases (type 3c). *Diabetes Metab Res Rev* 2012;28(4):338–42.
28. Cui YF, Andersen DK. Pancreatogenic diabetes: special considerations for management. *Pancreatology* 2011;11(3):279–94.
29. Mattar R, Lima GAS, da Costa MZG, Silva-Etto JMK, Guarita D, Carrilho FJ. Comparison of fecal elastase 1 for exocrine pancreatic insufficiency evaluation between ex-alcoholics and chronic pancreatitis in patients. *Arq Gastroenterol* 2014;51:297–301.
30. Creutzfeldt W, Gleichmann D, Otto J, Stöckmann F, Maisonneuve P, Lankisch PG. Follow-up of exocrine pancreatic function in type-1 diabetes mellitus. *Digestion* 2005;72:71–5.
31. Teichmann J, Riemann JF, Lange U. Prevalence of exocrine panceatic insufficiency in women with obesity syndrome: assessment by pancreatic fecal elastase 1. *ISRN Gastroenterol* 2011; 2011:951686. doi: 10.5402/2011/951686.
32. Nakajima K, Nemoto T, Muneyuki T, Kakei M, Fuchigami H, Munakata H. Low serum amylase in association with metabolic syndrome and diabetes: A community-based study. *Cardiovasc Diabetol* 2011;10(34):1–8.
33. Swislocki A, Noth R, Hallstone A, Kyger E, Triadafilopoulos G. Secretin-stimulated amylase release into blood is impaired in type 1 diabetes mellitus. *Horm Metab Res* 2005;37(5):326–30.
34. Aughsteen A, Abu-Umair MS, Mahmoud SA. Biochemical analysis of serum pancreatic amylase and lipase enzymes in patients with type 1 and type 2 diabetes mellitus. *Saudi Med J* 2005;26(1):73–7.
35. Basso D, Fabris C, Meani A, Del Favero G, Vianello D, Angonese C, et al. C reactive protein in pancreatic cancer and chronic pancreatitis. *Ann Clin Res* 1988;20(6):414–16.
36. Afzal S, Bojesen S, Nordestgaard BG. Low 25-hydroxyvitamin D and risk of type 2 diabetes: a prospective cohort study and metaanalysis. *Clin Chem.* 2013;59(2):381–91.
37. Danescu LG, Levy S, Levy J. Vitamin D and diabetes mellitus. *Endocrine* 2009; 35:11–7.
38. Isaia G, Giorgino R, Adami S. High prevalence of hypovitaminosis D in female type 2 diabetic population. *Diabetes Care* 2001;24(8):1496.
39. Di Cesar DJ, Ploutz-Snyder R, Weinstock RS, Moses AM. 25(OH)-vitamin D deficiency is more common in type 2 than in type 1 diabetes. *Diabetes Care* 2006;29(1):174.
40. Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, et al. Adiposity in relation to vitamin D status and parathyroid hormone levels: A population-based study in older men and women. *J Clin Endocrinol Metab* 2005;90:4119–23.
41. Ding C, Gao D, Wilding J, Trayhurn P, Bing C. Vitamin D signalling in adipose tissue. *Br J Nutr* 2012;108(11):1915–23.
42. Montagnani A, Gonnelli S, Alessandri M, Nuti R. Osteoporosis and risk of fracture in patients with diabetes: an update. *Aging Clin Exp Res* 2011;23(2):84–90.

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